Social Communication in Young Children with Sex Chromosome Trisomy

Evelien Urbanus

Social Communication in Young Children with Sex Chromosome Trisomy: Neurocognitive Building Blocks of Behavioral Outcomes

Evelien Urbanus

Evelien Urbanus

Social communication in young children with sex chromosome trisomy: Neurocognitive building blocks of behavioral outcomes

Leiden University

Faculty of Social and Behavioural Sciences

Clinical Neurodevelopmental Sciences

Cover design and printing by Ridderprint (www.ridderprint.nl)

The research reported in this dissertation was funded by:

Nederlandse Organisatie voor Wetenschappelijk Onderzoek

Social Communication in Young Children with Sex Chromosome Trisomy:

Neurocognitive Building Blocks of Behavioral Outcomes

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op dinsdag 6 september 2022
klokke 13.45 uur

door

Eva Laura Urbanus

geboren te Zoetermeer in 1991

Promotores

Dr. S. (Sophie) van Rijn

Prof.dr. H. (Hanna) Swaab

Promotiecommissie

Prof.dr. L. R. A. (Lenneke) Alink	Wetenschappelijk Directeur Instituut Pedagogische Wetenschappen/voorzitter
Prof.dr. P. W. (Paul) van den Broek	
Prof.dr. M. V. (Maretha) de Jonge	
Prof.dr. L. (Lydia) Krabbendam	Vrije Universiteit Amsterdam
Prof.dr. W. G. (Wouter) Staal	Radboud UMC Nijmegen
Prof.dr. C. T. R. M. (Constance) Stumpel	UMC Maastricht

Table of contents

Chapter 1

General Introduction

To gain a better understanding of neurodevelopmental problems, researchers traditionally have focused on individuals with a behavioral diagnosis, such as autism spectrum disorder (ASD) or attention deficit hyperactivity disorder (ADHD), to search for neurocognitive mechanisms underpinning behavioral outcomes. This approach has provided essential knowledge about the etiology of neurodevelopmental problems, however there are two major drawbacks. First, although individuals may have the same behavioral diagnosis, the underlying pathways leading to this diagnosis can be diverse. This makes it difficult to draw conclusions concerning causality. Second, neurodevelopmental impact can only be diagnosed in behavioral outcomes once a clinical behavioral classification is made, which typically is preceded by a cascade of neurodevelopmental problems leading towards the diagnosis and incorporates environmental influences as well. For example, the mean age at diagnosis for ASD is 43 months (van 't Hof et al., 2021), and ADHD is typically diagnosed between 7-9 years of age (Kessler et al., 2007). Due to this 'delay', knowledge about the early development of individuals with behavioral disorders is often based on retrospective information. As a consequence, critical windows of opportunity to intervene might have been missed.

Over the last decades, researchers have started to use a complementary approach to study neurobehavioral phenotypes to learn about neurodevelopmental mechanisms; namely by studying populations with neurobehavioral phenotypes associated with a clear genetic cause. This *neurogenetic approach* can give valuable insight in which factors can explain or predict adverse outcomes (Reiss & Dant, 2003). Knowledge on neurobehavioral phenotypes associated with genetic disorders is necessary to ultimately understand and identify individual patterns of development (Baumgardner et al., 1994; Sroufe & Rutter, 1984). As many genetic disorders can be detected prenatally, this presents the opportunity to study developmental pathways prospectively. Results and implications from studies including individuals with genetic disorders will not only benefit individuals with the genetic disorder but can also serve as a model of behavioral and cognitive conditions that affect individuals in the absence of a genetic disorder (Reiss et al., 2000).

A genetic disorder that could serve as a model for identification of neurodevelopmental mechanisms driving the increased risk for neurobehavioral problems and psychopathology should – preferably – meet certain criteria: 1) The genetic disorder should not be rare, 2) Global intellectual functioning should not be (severely) impaired as this would hinder drawing conclusions, 3) Prenatal or early diagnosis is possible, providing the opportunity to prospectively investigate the early developmental impact, and 4) The genetic disorder has a clear link with neurodevelopmental problems and/or psychopathology.

Sex chromosome trisomy (SCT) is a class of genetic disorders that meets these criteria. Typically, humans are born with 46 chromosomes: 22 pairs of autosomes and two sex chromosomes (XX in females and XY in males), resulting in a 46,XX or 46,XY karyotype. Due to a *de novo* non-disjunction during early cell division, the genetic make-up can contain an extra X or Y chromosome. This leads to a 47,XXY (Klinefelter syndrome) or 47,XYY (XYY syndrome) chromosomal pattern in males, and a 47,XXX (Trisomy X syndrome) in females. Regarding the four criteria mentioned above, SCT meets these criteria as: 1) SCT is one of the most common genetic duplications in humans with an estimated prevalence ranging from

1:650-1:1000 live births, 2) Although slightly lowered, global intellectual functioning is typically within the normal ranges, 3) SCT can be diagnosed prenatally, and 4) The X and Y chromosomes play an important role in neurodevelopment and the prevalence of neurodevelopmental disorders is increased in the SCT population.

Taken together, SCT could serve as a valuable model to study neurocognitive mechanisms driving neurodevelopmental problems and increased risk for psychopathology. Within the next paragraphs of this introduction, we will provide a short overview of the knowledge on SCT. Next, the importance of the X and Y chromosomes for neurodevelopment will be discussed. Third, from a bottom-up perspective, we will look into how SCT can inform us about mechanisms driving neurodevelopmental risk, with a specific focus on the communication domain as a building block for neurobehavioral outcomes. Lastly, the importance of studying young children will be illustrated before the aims and outlines of the dissertation will be discussed.

Trisomy of the X or Y Chromosomes

Within the literature, the vast majority of studies on SCT have focused on physical and medical consequences. For example, individuals with SCT are known to have a tall stature after puberty, hypotonia or low muscle tone is common, and infertility is found in males with an extra X (for a review see Tartaglia et al., 2020). Studies focusing on neurocognitive or behavioral outcomes, however, are rare and especially knowledge of early development is lacking. Before 1970, only a handful of studies investigated SCT, as genetic testing was reserved for individuals with severe physical dysmorphisms and/or psychological problems. Therefore, only severe cases were included in clinical descriptions of individuals with SCT. From the 1970's to 1990's seven research sites across the United States, Canada, and Europe used newborn screening protocols to identify children with X or Y chromosomal variations. The core knowledge of how SCT impacts neurocognitive and behavioral outcomes is based on these birth cohort studies (for a summary see Robinson et al., 1990). Identified children were followed until young adulthood, providing unbiased and prospective information on how SCT impacts development. Although these studies provide valuable information, most of these studies had a descriptive nature. In addition, the strength of the conclusions of these studies was limited by the size of the included samples, subsequently also limiting the opportunity to explore potential moderating variables, such as the impact of an extra X versus an extra Y.

Although SCT is a relatively common genetic variation which can be diagnosed before birth, historically only about 10% of individuals received the diagnosis before adolescence (Abramsky & Chapple, 1997; Bojesen et al., 2003). There are several possible explanations for this underdiagnosis. For example, the physical consequences of SCT can be relatively subtle and the impact of SCT on neurocognitive and behavioral outcomes is variable. These subtle physical characteristics and variability in symptoms do not often prompt genetic testing. Consequently, individuals may be treated for symptoms without knowledge of the underlying genetic condition. Due to advances in the technology to detect genetic variations in unborn children over the past years (e.g., non-invasive methods such as the screening of maternal blood), an exponential increase of prenatally diagnosed individuals with SCT is expected. To better serve the SCT population, more in-depth knowledge of the neurocognitive and behavioral

consequences of an extra sex chromosome is warranted and the advances in prenatal screening methods provide the opportunity to study children from a young age.

The Importance of the X and Y Chromosomes for Neurodevelopment

The X chromosome plays an important role in typical brain development and in the development of human intelligence (Johnson et al., 2009). The frequency of genes that affect general cognitive ability is 3.5 times higher on the X chromosome compared to any of the autosome genes, which makes the X chromosome disproportionally important for cognitive ability (Zechner et al., 2001). Genes on the Y chromosome have also been identified to play a role in brain development, independent from the X chromosome (Berletch et al., 2015). The gene density on the X chromosome is much higher than on the Y chromosome; the X chromosome contains approximately 800-900 coding genes, whereas the Y chromosome contains approximately 60-70 coding genes (ensembl.org; see Figure 1). To maintain relative equivalence in gene dosage between males with a 46,XY chromosomal pattern and females with a 46,XX chromosomal pattern, only one X chromosome is typically activated in females. Approximately 15% of the genes located on the X chromosome however, 'escape' inactivation (Carrel & Willard, 2005), these genes are then expressed in excess. In addition, there are genes on the Y chromosome that have identical homologous regions on the X chromosome, for example the X-chromosomal pseudoautosomal regions that escape inactivation in females are also present on the Y chromosome (see Figure 1). These genes that are located on both the X and Y chromosome, for example neuroligin, may play a significant role in the etiology of autism and other communication disorders (Bishop & Scerif, 2011). The importance of the X and Y chromosomes for neurodevelopment and the link between the X and Y chromosomes in combination with the risk for psychopathology make the X and Y chromosomes interesting candidates to study how genetic make-up in interaction with the environment can lead to behavioral outcomes.

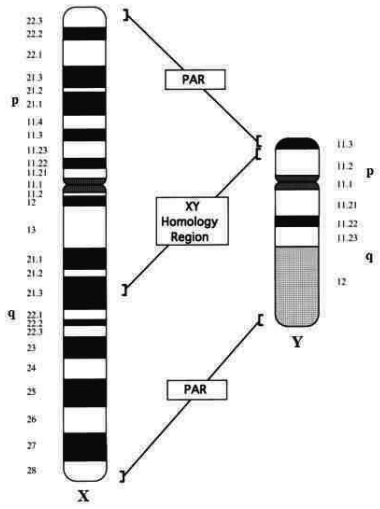

Figure 1. The X and Y chromosomes. Figure adapted from Mumm et al. (1997)

How SCT Can Inform Us About Mechanisms Driving Neurodevelopmental Risk

A valuable model to describe how genetic make-up in interaction with environmental factors can lead to behavioral outcomes, is the brain behavioral model (Figure 2). This model uses a bottom-up approach to explain how an individual's genetic make-up is reflected in both the architecture of the brain and the functioning of the brain. Neurocognitive functions are the expression of the architecture and functioning of the brain and reflect the ability to process information. A complex interplay of multiple neurocognitive functions results in behavior, thus, neurocognitive functions are the building blocks for behavioral outcomes. Environmental factors can influence all levels of the model (Swaab et al., 2011).

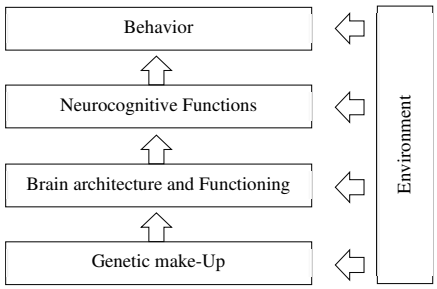

Figure 2. The brain behavioral model from a bottom-up approach

Starting at the bottom when applying the brain behavioral model to SCT, the genetic make-up, or the presence of an extra X or Y chromosome, causes an excess of expression of genes that are important for neural development and related neurocognitive functions (Lenroot et al., 2014; Raznahan et al., 2016). Consequently, the brain behavioral model indicates that the presence of the extra X or Y chromosome impacts the development of the brain.

Neuroimaging studies have researched the effect of SCT on both the structure or architecture of the brain and on brain functioning. Regarding structural effects, studies show that the presence of an extra X or Y chromosome has both convergent and dissociable effects on the anatomy of the brain. Overall, it appears that an extra X chromosome in males and females leads to a decreased total brain volume, whereas the presence of an extra Y chromosome in males leads to an increased total brain volume (Bryant et al., 2012; Raznahan et al., 2016). In addition to an impacted overall brain volume, multiple studies have reported thinning in the (lateral) temporal and frontal brain regions, including subcortical structures such as the amygdala, insula, hippocampus, and cingulate gyrus (Giedd et al., 2007; Lenroot et al., 2014; Lentini et al., 2013; Nadig et al., 2018; Patwardhan et al., 2002; Warling et al., 2020). Researchers have reported mixed results regarding the occipital and parietal regions of the brain; some researchers indicate that these regions are affected as well (Warling et al., 2020), whereas others find that these regions are preserved (Giedd et al., 2007; Lenroot et al., 2014). The effects of SCT on brain functioning have been studied to a lesser extent, and studies mostly included only males with XXY. In addition, studies used different designs, different functional imaging techniques, and included participants from various ages, limiting the ability to compare outcomes. A few studies have investigated language lateralization, and mixed results ranging

from no differences to altered lateralization have been reported (van Rijn et al., 2008; Wallentin et al., 2016; Wilson & Bishop, 2018). Two studies have investigated amygdala activation during exposure to facial expressions and both report contrasting results, ranging from reduced amygdala activation to a tendency for increased amygdala activation (Brandenburg-Goddard et al., 2014; van Rijn et al., 2012). Lastly, one study reported no differences in frontal brain activity when presented with a Stroop task (Wallentin et al., 2016). Although studies have not yet provided evidence to support a direct link between the affected brain regions and neurocognitive functioning in individuals with SCT (Skakkebaek et al., 2020), it is likely that the neurocognitive difficulties experienced by this population are anchored in the brain.

Due to the impact of the X and Y chromosomes on multiple brain regions, several information processing functions can be affected, leading to a range of neurocognitive difficulties encompassing various domains of functioning. It is important to identify neurocognitive strengths and weaknesses in this genetic population, and to link this profile of strengths and weaknesses to behavioral outcomes to learn about underlying neurocognitive mechanisms that drive these outcomes.

At the top level of the brain behavioral model, the behavioral level, an increased risk for neurodevelopmental disorders has been reported. For example, estimates of the prevalence of ASD range from 18-30% in children with SCT (Van Rijn, 2019) versus 0.6% in the general population (Elsabbagh et al., 2012). For ADHD, 25-43% of the children with SCT meets the diagnostic criteria (Van Rijn, 2019), versus 7.2% in the general population (Thomas et al., 2015). Higher rates for other forms of psychopathology have been reported as well. For example, the risk for schizophrenia and bipolar disorder has been estimated to be three to four times higher for individuals with SCT (Bardsley et al., 2013; Cederlöf et al., 2014; Wigby et al., 2016). Lastly, studies have hinted at an increased risk for depression and anxiety disorders, in particular in individuals with an extra X chromosome (for an overview see Green et al., 2019).

Taken together, the presence of an extra X or Y chromosome impacts the brain, which subsequently impacts the neurocognitive functions that act as building blocks for behavioral outcomes. By gaining knowledge of these neurocognitive underpinnings of behavior, diagnostic assessment and treatment may improve, not only for the SCT population but ultimately for the general population as well as this knowledge could help focus on relevant domains of individual functioning in assessment. Furthermore, identifying which neurocognitive building blocks are important for specific behavioral outcomes is essential as focusing on specific targets for intervention may mitigate developmental impact by enabling more tailored mental health care.

Building Blocks for Neurobehavioral Outcomes in the Domain of Communication

Neurobehavioral outcomes in the SCT population are diverse. Knowledge of which neurocognitive building blocks are associated with specific behavioral outcomes is essential. Although neurocognitive vulnerabilities have been identified on several domains, the focus of this dissertation will be on the communication domain. Within the next paragraphs we will

discuss the concept of communication, the importance of communicative abilities in relation to the risk for psychopathology, and the importance of studying communication in the SCT population.

Communication is the process of information exchange between individuals, reflecting a person's ideas, thoughts, feelings, needs, or desires. There are different kinds of modalities someone can use to communicate, including verbal communication, written communication, and the use of gestures (Levey, 2019). Communication is an active process involving the exchange of information between a sender and a receiver; the sender transmits or encodes information that the receiver decodes to comprehend or understand (Owens Jr., 2011). Within the communication domain, several components can be identified. These include speech, language, the use of paralinguistic cues such as intonation and volume, and the use of nonlinguistic cues such as facial expression and posture (Levey, 2019). The degree to which someone is successful in communicating, measured by the appropriateness and effectiveness of sending and receiving messages, is called communicative competence (Hymes, 1972).

The development of communication starts before children are born. When babies are around 24 weeks gestational age, they can hear sounds and they familiarize with voices they hear often. After birth, communication develops further. Although the human brain is prewired for communication, early learning is of great importance and the social basis for communicative development starts within the mother-child dyad. Babies are typically fascinated by faces and voices, showing a marked preference for faces over inanimate objects. When children are only one month old, they will respond to their mothers' vocalizations by making eye contact and following direction of gaze. Within the first months of life, babies continue to learn, for example by paying attention to what they hear and observe in their surroundings. During this time, babies communicate by crying, using different types and intensities of cries to express different needs. Around 4-6 months, babies start to communicate with more vocalizations and babbling. Accompanied by improving motor skills such as the ability to sit and later crawl, children gain the ability to further explore the world. Desired objects or people can be too far away and, in addition to babbling, children communicate their intentions by the use of gestures, such as showing or pointing. By the age of one, most children start to understand the meaning of words and create a verbal understanding. Around 18 months most children start to use spoken language themselves. The number of words a child understands (i.e., receptive vocabulary) and the number of words a child uses (i.e., expressive vocabulary) expands tremendously in a short time period. On average, children have an expressive vocabulary of 20 words around 18 months, which increases to 200-300 words at 24 months, and 2000 words at the age of 5 years (Owens Jr., 2011). In addition, children start to create sentences, combining two words between the ages of 1 and 2 years, and combining three to four words around the age of 3 years. Over the years, sentences will contain more words and become more grammatically complex (i.e., development of syntax), around 7-8 years, most children are able to use complex sentence structures (Simms, 2007). Conversational skills, such as turn taking or maintaining a topic, are refined during the school years. Although at a slower pace, language and communication skills will continue to develop during late childhood, adolescence, and adulthood.

In addition to the development of verbal communication skills, other communicative functions, such as nonverbal communication and conversational skills, continue to develop as well, in particular during social interaction. During social interactions, spoken messages are often accompanied by nonverbal communicative cues, such as facial expression, intonation, or prosody. These nonverbal communicative cues help convey a speaker's intentions or help the receiver to understand the meaning, furthering communicative competence.

Communicative competence is fundamental to successfully participate in society (Rickheit et al., 2008). It is a foundation skill for life and an important building block for many other aspects of life, including social interaction, reflecting on one's own behavior, and behavioral regulation. Language and communication are crucial for further cognitive and social development (Simms, 2007). For that reason, it is not surprising that difficulties with communication are associated with adverse behavioral outcomes and neurodevelopmental problems (Gallagher, 1999).

Within the SCT population, difficulties with language are considered one of the most distinctive traits. Studies have reported language and communication difficulties in as many as 80% of included individuals (Boada et al., 2009; Leggett et al., 2010; Robinson et al., 1983). It should be noted that the method of examining what would be considered as 'difficulty' varies between studies. When reporting outcomes, studies often do not only include specific language and communication measures, but other measures as well. These include, but are not limited to, speech assessments, verbal intelligence, and school reports. In addition, within this percentage, rates of individuals that have received speech- or language therapy, or with language-based learning problems have been included as well. Based on the current literature however, there are two main gaps in the knowledge of language and communication development in SCT. First, the focus of studies investigating language outcomes has been on school-aged children, adolescents, and/or adults. Only a handful of studies – often including only small samples – has included young children. Second, studies that have included specific language outcomes have primarily focused on structural language, including the form and content of language, whereas the impact of SCT on the use of language in a social context and on the broader communication domain has been understudied.

Importance of Studying Communication in Early Child Development

As communication starts to develop from a very young age and develops rapidly in the first years of life (Simms, 2007) and as difficulties with communication at an early age can be a precursor for later neurodevelopmental problems, it is striking that there is little knowledge of the early language and communicative development of children with SCT.

This lack of knowledge of the communicative development of young children in combination with the expected increase in prenatal diagnoses stresses the importance of research in this area. Knowledge about early development could help pinpoint which communicative abilities are vulnerable; for example, if there are difficulties in the communication domain that extend past the recognized risk for structural language difficulties that have been reported in older individuals. In addition to pinpointing vulnerabilities, the opportunity to study a group of children with a clear genetic disorder from birth offers the

unique ability to investigate developmental pathways and possible underlying mechanisms for later outcomes. For example, knowledge about the early development could help identify precursors and early markers for later adverse outcomes, such as behaviors associated with ASD, ADHD, or other psychopathology. By studying these abilities from a developmental perspective, windows of opportunity to support development could be identified. This is not only informative for individuals with SCT but could also increase the understanding of development and developmental risk in the general population. Lastly, this knowledge is needed for clinical purposes; to further inform parents, genetic counselors, pediatricians, developmental psychologists, and all other involved parties on the range of outcomes associated with SCT. Important questions that need to be answered include What are developmental strengths and weaknesses? Which domains are important to monitor? Which abilities could be important targets for early support or intervention?

Thus, we are in need of more knowledge on the early development of children with SCT. Knowledge of these early communicative abilities will help determine which abilities could serve as important targets for early treatment and intervention that could potentially influence the developmental trajectory of young children with SCT in a positive manner.

TRIXY Early Childhood Study

The TRIXY Early Childhood Study is a longitudinal study that was developed to identify neurodevelopmental risks in young children with an extra X or Y chromosome. One of the aims of the study is to gain understanding of the early development of language and communication abilities. The TRIXY Early Childhood Study is based at the TRIXY Center of Expertise in Leiden, the Netherlands, with multiple national and international recruitment and testing sites, including the eXtraordinarY Kids Clinic, Children's Hospital Colorado. Participants in the study are children between 1-7 years old (SCT or control) and their primary caregiver.

Children with SCT were recruited with the help of clinical genetic departments, pediatricians, and national advocacy or support groups in the Netherlands, Colorado USA, and Belgium. For all children in the SCT group, presence of the trisomy ($\geq$ 80% of the cells) was confirmed by requesting the karyotyping outcomes performed by academic hospitals. Children within the same age-range were recruited in the Western parts of the Netherlands to take part as a control group. Due to ethical reasons, genetic screening was not performed in the control group. However, based on the SCT prevalence, the risk of including a child with SCT in the control group was considered minimal and acceptable. All included children and their primary caregiver had to understand Dutch or English. Children with a history of traumatic brain injury, severely impaired hearing or sight, neurological illness, or colorblindness were excluded from the study.

Within the longitudinal design of the TRIXY Early Childhood Study, children were seen during an initial baseline assessment and a follow-up took place approximately 12 months later. Children in the SCT group were included regardless of SCT karyotype (XXX, XXY, XYY), time of diagnosis (prenatal, postnatal), or ascertainment site (i.e., the reason for enrollment in the study). Not selecting on these factors allowed us to determine if specific subgroups of children with SCT have an added risk for unfavorable outcomes. Within each paper, via

preliminary analyses or specific research questions we consider the question if SCT karyotype, time of diagnosis, and ascertainment bias are relevant factors for the interpretation of the results.

In total, 209 children where included: 107 children with SCT and 102 age-matched population controls. At recruitment the age of the children ranged from 11 months to 7 years and 8 months. Within the SCT group, 33 girls with XXX, 50 boys with XXY, and 24 boys with XYY were included. Seventy-two children had a prenatal diagnosis (67%). Reasons for enrollment in the study ('ascertainment bias') were categorized into one of three categories: 'Prospective follow-up', including children with a prenatal diagnosis who are actively followed over time (51%), 'information seeking', including families who want to learn more about their child's condition, but without specific concerns of their child's development (30%), or 'clinically referred cases', including children receiving professional help or from families with specific developmental concerns (19%). Within the control group 58 girls and 44 boys were included.

Aims and Outline of this Dissertation

The central aim of this dissertation is to study early language abilities of young children with SCT within the broader communication domain and to prospectively investigate the relationship between communication and behavioral outcomes. More specifically, within this dissertation we aim to gain knowledge of the behavioral profile, structural language abilities, pragmatic language abilities, and attention and responses to short communicative interactions, to understand mechanisms that may help explain developmental risk and behavioral outcome and to identify targets for early interventions.

Previous studies indicate that individuals with SCT have an elevated risk for serious behavioral difficulties. It is possible that early signs of these behavioral difficulties emerge when children are younger; the developing brain could give more insight on when psychopathology emerges and how it unfolds (Andersen, 2003). Studies including young children, however, are scarce whereas this knowledge is particularly important to identify children who are at risk for more serious neurodevelopmental disorders as early in life as possible and to help reduce the risk for behavioral dysfunction later in life. In **Chapter 2** we assess the profile of 1-year-old, 2-3-year-old, and 4-5-year-old children with SCT on the following behavioral outcomes: Affective problems, anxiety, pervasive developmental problems, attention deficit problems, oppositional defiant problems, and social-emotional functioning. In addition to knowledge of behavioral outcomes, it is important to focus on neurocognitive underpinnings of behavior, as behavioral problems may arise as a consequence of different information processing deficits. Therefore, evidence for impairments in the domains of global intellectual functioning, language, executive functioning, and social cognition are evaluated through a narrative review in **Chapter 3**, with a focus on early development. Within the next chapters of this dissertation, the focus will be on the domain of language and communication as possible building blocks for behavioral outcomes.

Earlier studies including school-aged children, adolescents, and/or adults indicate that a high percentage (70-80%) of individuals with SCT experiences some form of language difficulty (Boada et al., 2009; Leggett et al., 2010; Robinson et al., 1983). Less is known

however, about the first few years of language development, which is striking as language develops rapidly at this age due to significant brain growth. For that reason, this developmental perspective is included in chapters 4, 5, and 6. In **Chapter 4** we focus on language abilities of children with SCT between 1-6 years; a time when several important milestones within child development occur. The time between the ages of 1 to 6 years comprises the period where children rely mostly on nonverbal communication to the period where children begin to use words and finally to a period where children start learning more complex forms of language. With various language measurements the use of gestures, early vocabulary, receptive semantics, expressive semantics, syntax, and phonological processing skills are evaluated at different developmental stages. In addition to the 'structural language measures' related to the *form* and *content* of language that were evaluated in **Chapter 4**, the *use* of language in a social context or pragmatic language is also an important factor in social interaction and communication. In **Chapter 5** we evaluate if the presence of an extra X or Y not only affects structural language, but also pragmatic language. In other words, the question whether there is a broader communication deficit, that extends past the structural language difficulties is addressed. Secondly, in **Chapter 5** we explore if structural and pragmatic language abilities serve as building blocks for behavioral outcomes one year later and we aim to identify targets for early intervention in the communication domain. In **Chapter 6** we further explore the broader communicative abilities of young children with SCT. With eye tracking and physiological arousal measures we objectively assess how children respond to short communicative interactions. Several questions are addressed; which information do children attend to and which information do they miss? Does the direction of gaze during the interaction play a role in this? Do children modulate their arousal levels in reaction to different communicational demands? How do these broader communicative skills relate to structural language abilities? To evaluate to what degree social orientation and physiological arousal levels are related to real-life social behavior and to gain insight in underpinnings of social behavior, we included a group of typically developing children aged 3-7 years in **Chapter 7**. In this chapter, we explore how social orientation as measured with eye tracking relates to daily life behavior. Finally, in **Chapter 8** the conclusions and implications of the studies are summarized and discussed, and directions for future research are presented.

References

Abramsky, L., & Chapple, J. (1997). 47,XXY (Klinefelter syndrome) and 47,XYY: Estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenatal Diagnosis*, *17*(4), 363-368. https://doi.org/10.1002/(sici)1097-0223(199704)17:4%3C363::aid-pd79%3E3.0.co;2-o

Andersen, S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews*, *27*, 3-18. https://doi.org/doi:10.1016/S0149-7634(03)00005-8

Bardsley, M. Z., Kowal, K., Levy, C., Gosek, A., Ayari, N., Tartaglia, N., . . . Ross, J. L. (2013). 47,XYY syndrome: Clinical phenotype and timing of ascertainment. *The Journal of Pediatrics*, *163*(4), 1085-1094. https://doi.org/10.1016/j.jpeds.2013.05.037

Baumgardner, T. L., Green, K. E., & Reiss, A. L. (1994). A behavioral neurogenetics approach to developmental disabilities: Gene-brain-behavior associations. *Current Opinion in Neurology*, *7*, 172-178. https://doi.org/10.1097/00019052-199404000-00015

Berletch, J. B., Ma, W., Yang, F., Shendure, J., Noble, W. S., Disteche, C. M., & Deng, X. (2015). Escape from X inactivation varies in mouse tissues. *PLoS Genetics*, *11*(3), Article e1005079. https://doi.org/10.1371/journal.pgen.1005079

Bishop, D. V., & Scerif, G. (2011). Klinefelter syndrome as a window on the aetiology of language and communication impairments in children: the neuroligin-neurexin hypothesis. *Acta Paediatrica*, *100*(6), 903-907. https://doi.org/10.1111/j.1651-2227.2011.02150.x

Boada, R., Janusz, J., Hutaff-Lee, C., & Tartaglia, N. (2009). The cognitive phenotype in Klinefelter syndrome: A review of the literature including genetic and hormonal factors. *Developmental Disabilities Research Reviews*, *15*(4), 284-294. https://doi.org/10.1002/ddrr.83

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevelance of Klinefelter syndrome: A national registry study. *The Journal of Clinical Endocrinology & Metabolism*, *88*(2), 622-626. https://doi.org/10.1210/jc.2002-021491

Brandenburg-Goddard, M. N., Van Rijn, S., Rombouts, S., Veer, I. M., & Swaab, H. (2014). A comparison of neural correlates underlying social cognition in Klinefelter syndrome and autism. *Social Cognitive and Affective Neuroscience*, *9*(12), 1926-1933. https://doi.org/10.1093/scan/nst190

Bryant, D. M., Hoeft, F., Lai, S., Lackey, J., Roeltgen, D., Ross, J., & Reiss, A. L. (2012). Sex chromosomes and the brain: A study of neuroanatomy in XYY syndrome. *Developmental Medicine and Child Neurology*, *54*(12), 1149-1156. https://doi.org/10.1111/j.1469-8749.2012.04418.x

Carrel, L., & Willard, H. F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature*, *434*(7031), 400-404. https://doi.org/10.1038/nature03479

Cederlöf, M., Ohlsson Gotby, A., Larsson, H., Serlachius, E., Boman, M., Långström, N., . . . Lichtenstein, P. (2014). Klinefelter syndrome and risk of psychosis, autism, and ADHD. *Journal of Psychiatric Research*, *48*(1), 128-130. https://doi.org/10.1016/j.jpsychires.2013.10.001

Elsabbagh, M., Divan, G., Koh, Y. J., Kim, Y. S., Kauchali, S., Marcín, C., . . . Fombonne, E. (2012). Global prevalence of autism and other pervasive developmental disorders. *Autism Research: Official Journal of the International Society for Autism Research*, *5*(3), 160-179. https://doi.org/10.1002/aur.239

Gallagher, T. M. (1999). Interrelationships among children's language, behavior, and emotional problems. *Topics in Language Disorders*, *19*(2), 1-15. https://doi.org/10.1097/00011363-199902000-00003

Giedd, J. N., Clasen, L. S., Wallace, G. L., Lenroot, R. K., Lerch, J. P., Wells, E. M., . . . Samango-Sprouse, C. A. (2007). XXY (Klinefelter syndrome): A pediatric quantitative brain magnetic resonance imaging case-control study. *Pediatrics*, *119*(1), e232-240. https://doi.org/10.1542/peds.2005-2969

Green, T., Flash, S., & Reiss, A. L. (2019). Sex differences in psychiatric disorders: What we can learn from sex chromsome aneuploidies. *Neuropsychopharmacology*, *44*, 9-21. https://doi.org/10.1038/s41386-018-0153-2

Hymes, D. (1972). On communicative competence. In I. B. Pride & J. Holmes (Eds.), *Sociolinguistics* (pp. 269-293). Penguin Bookds Ltd.

Johnson, W., Carothers, A., & Deary, I. J. (2009). A role for the X chromosome in sex differences in variablity in general intelligence? *Perspectives on Psychological Science*, *4*(6), 598-611. https://doi.org/10.1111/j.1745-6924.2009.01168.x

Kessler, R. C., Amminger, G. P., Aguilar-Gaxiola, S., Alonso, J., Lee, S., & Üstün, T. B. (2007). Age of onset of mental disorders: A review of recent literature. *Current Opinion in Psychiatry*, *20*(4), 359-364. https://doi.org/10.1097/YCO.0b013e32816ebc8c

Leggett, V., Jacobs, P., Nation, K., Scerif, G., & Bishop, D. V. (2010). Neurocognitive outcomes of individuals with a sex chromosome trisomy: XXX, XYY, or XXY: A systematic review. *Developmental Medicine & Child Neurology*, *52*(2), 119-129. https://doi.org/10.1111/j.1469-8749.2009.03545.x

Lenroot, R. K., Blumenthal, J. D., Wallace, G. L., Clasen, L. S., Lee, N. R., & Giedd, J. N. (2014). A case-control study of brain structure and behavioral characteristics in 47,XXX syndrome. *Genes Brain and Behavior*, *13*(8), 841-849. https://doi.org/10.1111/gbb.12180

Lentini, E., Kasahara, M., Arver, S., & Savic, I. (2013). Sex differences in the human brain and the impact of sex chromosomes and sex hormones. *Cerebral Cortex*, *23*(10), 2322-2336. https://doi.org/10.1093/cercor/bhs222

Levey, S. (2019). *An introduction to language development, Second edition*. Plural Publishing, Inc., San Diego

Mumm, S., Molini, B., Terell, J., Srivastava, A., & Schlessinger, D. (1997). Evolutionary features of the 4-Mb Xq21.3 XY homology region revealed by a map at 60-kb resolution. *Genome Research*, *7*(4), 307-314. https://doi.org/10.1101/gr.7.4.307

Nadig, A., Reardon, P. K., Seidlitz, J., McDermott, C. L., Blumenthal, J. D., Clasen, L. S., . . . Raznahan, A. (2018). Carriage of supernumerary sex chromosomes decreases the volume and alters the shape of limbic structures. *eNeuro*, *5*(5). https://doi.org/10.1523/ENEURO.0265-18.2018

Owens Jr., R. E. (2011). *Language development: An introduction* (8th ed., International ed. ed.). Pearson Education.

Patwardhan, A. J., Brown, W. E., Bender, B. G., Linden, M. G., Eliez, S., & Reiss, A. L. (2002). Reduced size of the amygdala in individuals with 47,XXY and 47,XXX karyotypes. *American Journal of Medical Genetics (Neuropsychiatric Genetics)*, *114*(1), 93-98. https://doi.org/10.1002/ajmg.10154

Raznahan, A., Lee, N. R., Greenstein, D., Wallace, G. L., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2016). Globally divergent but locally convergent X- and Y-chromosome influences on cortical development. *Cerebral Cortex*, *26*, 70-79. https://doi.org/10.1093/cercor/bhu174

Reiss, A. L., & Dant, C. C. (2003). The behavioral neurogenetics of fragile X syndrome: Analyzing gene–brain–behavior relationships in child developmental psychopathologies. *Development and Psychopathology*, *15*, 927-968. https://10.1017.S0954579403000464

Reiss, A. L., Eliez, S., Schmitt, J. E., Patwardhan, A., & Haberecht, M. (2000). Brain imaging in neurogenetic conditions: Realizing the potential of behavioral neurogenetics research. *Mental Retardation and Developmental Disabilities Research Reviews*, *6*(3), 186-197. https://doi.org/10.1002/1098-2779(2000)6:3%3C186::AID-MRDD6%3E3.0.CO;2-9

Rickheit, G., Strohner, H., & Vorweg, C. (2008). The concept of communicative competence. In G. Rickheit & H. Strohner (Eds.), *Handbook of communication competence*. Mouton de Gruyter.

Robinson, A., Bender, B., Borelli, J., Puck, M., & Salbenblatt, J. (1983). Sex chromosomal anomalies: Prospective studies in children. *Behavior Genetics*, *13*(4), 321-329. https://doi.org/10.1007/BF01065770

Robinson, A., Bender, B. G., & Linden, M. G. (1990). Summary of clinical findings in children and young adults with sex chromosome anomalies. *Birth Defects Original Article Series*, *26*(4), 225-228.

Simms, M. D. (2007). Language disorders in children: Classification and clinical syndromes. *Pediatric Clinics of North America*, *54*, 437-467. https://doi.org/10.1016/j.pcl.2007.02.014

Skakkebaek, A., Gravholt, C. H., Chang, S., Moore, P. J., & Wallentin, M. (2020). Psychological functioning, brain morphology, and functional neuroimaging in Klinefelter syndrome. *American Journal of Medical Genetics part C*, *184*(2), 506-517. https://doi.org/10.1002/ajmg.c.31806

Sroufe, L. A., & Rutter, M. (1984). The domain of developmental psychopathology. *Child Development*, *55*(1), 17-29. https://doi.org/10.1111/j.1467-8624.1984.tb00271.x

Swaab, H., Bouma, A., Hendriksen, J., & König, C. (2011). *Klinische kinderneuropsychologie*. Uitgeverij Boom.

Tartaglia, N., Howell, S., Davis, S., Kowal, K., Tanda, T., Brown, M., . . . Ross, J. (2020). Early neurodevelopmental and medical profile in children with sex chromosome trisomies: Background for the prospective eXtraordinarY babies study to identify early risk factors and targets for intervention. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *184*(2), 428-443. https://doi.org/10.1002/ajmg.c.31807

Thomas, R., Sanders, S., Doust, J., Beller, E., & Glasziou, P. (2015). Prevalence of attention-deficit/hyperactivity disorder: A systematic review and meta-analysis. *Pediatrics*, *135*(4), e994-e1001. https://doi.org/10.1542/peds.2014-3482

van 't Hof, M., Tisseur, C., van Berckelear-Onnes, I., van Nieuwenhuyzen, A., Daniels, A. M., Deen, M., . . . Ester, W. A. (2021). Age at autism spectrum disorder diagnosis: A systematic review and meta-analysis from 2012 to 2019. *Autism*, *25*(4), 862-873. https://doi.org/10.1177/1362361320971107

Van Rijn, S. (2019). A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47,XXX, 47,XYY). *Current Opinion in Psychiatry*, *32*(2), 79-84. https://doi.org/10.1097/yco.0000000000000471

Van Rijn, S., Aleman, A., Swaab, H., Vink, M., Sommer, I., & Kahn, R. S. (2008). Effects of an extra X chromosome on language lateralization: An fMRI study with Klinefelter men (47,XXY). *Schizophrenia Research*, *101*(1-3), 17-25. https://doi.org/10.1016/j.schres.2008.02.001

Van Rijn, S., Swaab, H., Baas, D., de Haan, E., Kahn, R. S., & Aleman, A. (2012). Neural systems for social cognition in Klinefelter syndrome (47,XXY): Evidence from fMRI. *Social Cognitive and Affective Neuroscience*, *7*(6), 689-697. https://doi.org/10.1093/scan/nsr041

Wallentin, M., Skakkebaek, A., Bojesen, A., Fedder, J., Laurberg, P., Ostergaard, J. R., . . . Gravholt, C. H. (2016). Klinefelter syndrome has increased brain responses to auditory stimuli and motor output, but not to visual stimuli or Stroop adaptation. *Neuroimage-Clinical*, *11*, 239-251. https://doi.org/10.1016/j.nicl.2016.02.002

Warling, A., Liu, S., Wilson, K., Whitman, E., Lalonde, F. M., Clasen, L. S., . . . Raznahan, A. (2020). Sex chromosome aneuploidy alters the relationship between neuroanatomy and cognition. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *184*(2), 493-505. https://doi.org/10.1002/ajmg.c.31795

Wigby, K., D'Epagnier, C., Howell, S., Reicks, A., Wilson, R., Cordeiro, L., & Tartaglia, N. (2016). Expanding the phenotype of Triple X syndrome: A comparison of prenatal versus postnatal diagnosis. *American Journal of Medical Genetics Part A*, *170*(11), 2870-2881. https://doi.org/10.1002/ajmg.a.37688

Wilson, A. C., & Bishop, D. V. M. (2018). Sex chromosome trisomies are not associated with atypical lateralization for language. *Developmental Medicine and Child Neurology*, *60*(11), 1132-1139. https://doi.org/10.1111/dmcn.13929

Zechner, U., Wilda, M., Kehrer-Sawatzki, H., Vogel, W., Fundele, R., & Hameister, H. (2001). A high density of X-linked genes for general cognitive ability: A run-away process shaping human evolution? *TRENDS in Genetics*, *17*, 697-701. https://doi.org/10.1016/s0168-9525(01)02446-5

Chapter 2

The behavioral profile of children aged 1-5 years with sex chromosome trisomy (47,XXX, 47,XXY, 47,XYY)

Published as:

Urbanus, E., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2020). The behavioral profile of children aged 1-5 years with sex chromosome trisomy (47,XXX, 47,XXY, 47,XYY). *American Journal of Medical Genetics Part C: Seminars in Medical Genetics, 184*(2), 444-455. https://doi.org/10.1002/ajmg.c.31788

Abstract

Children with SCT have an increased risk of suboptimal neurodevelopment. Previous studies have shown an elevated risk for neurobehavioral problems in individuals with SCT. However, not much is known about neurobehavioral problems in very young children; knowledge that could help with early identification of children at risk for suboptimal development, and that could help establish targets for early intervention. This study addressed the question of what the behavioral profile of children with SCT aged 1–5 years looks like.

In total, 182 children aged 1–5 years participated in this study ($N_{SCT} = 87$, $N_{nonclinical\ controls} = 95$). Recruitment and assessment took place in the Netherlands and the United States. The SCT group was recruited through prospective follow-up (50%), information seeking parents (31%), and clinical referral (18%). Behavioral profiles were assessed with the child behavior checklist and the ages-and-stages social–emotional questionnaire.

Levels of parent-rated problem behavior were higher in children with SCT. Difficulties with overall social–emotional functioning were already present in 1-year-olds, and elevated scores were persistent across the full age range. Affective and pervasive developmental behaviors were seen in late toddlerhood and prominent at preschool age. Anxiety, attention deficit, and oppositional defiant behaviors were seen in preschool-aged children. Within this cross-sectional study, the developmental trajectory of affective, pervasive developmental, and oppositional defiant behaviors seemed to be different for SCT children than nonclinical controls.

Collectively, these results demonstrate the importance of behavioral screening for behavioral problems in routine clinical care for children with SCT from a young age. Social–emotional problems may require special attention, as these problems seem most prominent, showing increased risk across the full age range, and with these problems occurring regardless of the timing of diagnosis, and across all three SCT karyotypes.

Introduction

Sex chromosome trisomy (SCT; the presence of an extra X or Y chromosome) is one of the most common chromosomal duplications in humans, with an estimated prevalence from 1-650 to 1-1000 live births (Bojesen et al., 2003; Groth et al., 2013; Morris et al., 2008). Children with SCT have an increased risk of suboptimal neurodevelopment, including problems with language development, social cognition, and executive functioning (for a review see Urbanus et al., 2019). An increased risk for neurodevelopmental disorders, such as Autism Spectrum Disorder (ASD), and Attention Deficit Hyperactivity Disorder (ADHD) has been described in all subtypes of SCT (for a review see Van Rijn, 2019). Although there is overlap in developmental phenotypes, some behavioral and emotional difficulties are found to be more common for specific karyotypes. Examples include high levels of anxiety in girls and boys with an extra X chromosome (Tartaglia, Howell, et al., 2010; Verri et al., 2010), and high levels of impulsivity and externalizing behavior in boys with an extra Y chromosome (Hong & Reiss, 2014).

Most studies on impact of SCT on neurodevelopment have been conducted in school-aged children, adolescents, and adults, and have shown that individuals with SCT have an elevated risk for serious behavioral dysfunctions. It is likely that early signs of these behavioral challenges emerge when children are younger. However, we have very little knowledge about the behavior profile of young children with SCT, and the impact of SCT on neurodevelopment of toddlers and preschoolers. For that reason, this study aimed to describe the behavioral profile of children with SCT in a very early developmental stage.

It should be noted that while studies generally indicate increased risk for behavioral problems in SCT, it has also been indicated that the behavioral profile of individuals with SCT is highly variable (e.g., Ross et al., 2012; Samango-Sprouse et al., 2013; Tartaglia, Cordeiro, et al., 2010). Although SCT is associated with risk for behavioral problems and psychopathology, some individuals function without any problems. It is unknown which mechanisms modulate this variability. However, the developing brain could give more insight on when psychopathology emerges and how it unfolds (Andersen, 2003), and possibly the maturation of the brain could help explain the observed variability of outcomes in individuals with SCT.

It is also important to gain more knowledge about the behavioral profile, and possible early presentation of behavioral problems in young children with SCT, to allow for development of age-specific screening (e.g., to identify children who are at risk for more serious neurodevelopmental disorders as early in life as possible), and for development of treatment recommendations (i.e., identifying targets for intervention and preventive support)., Knowledge about the early behavioral profile of children with SCT can help reduce the risk of behavioral dysfunction later in life for children who are at risk for developing psychopathology.

Taken together, this study aimed to describe the social-emotional and behavioral profile of children aged 1 to 5 with SCT. Since these early stages of childhood are characterized by substantial developmental changes in the brain, we expect high variability within this age group. For that reason, we will not merely focus on mean group findings, but also aim to describe the variability within this age group with risk assessment (i.e., how many of the children score within borderline or clinical ranges). Our main focus will be on age-related presentation of the

behavioral phenotype, to evaluate if developmental impact can be found within this window of 1-5 years. Moreover, we were also interested to see if there is stability of symptoms over time within this age range. Secondary to these research questions, differences in behavior problems between children with SCT and nonclinical controls were compared by karyotypes (XXX vs XX, XYY vs XY, XYY vs XY). Also, since problem behavior might be associated with the reason of detection of the SCT, behavioral outcomes were compared between pre- and postnatally identified children, and the role of ascertainment was assessed.

Method

Participants

The present study is part of a larger ongoing project (the TRIXY Early Childhood Study), which includes children with SCT and nonclinical controls aged 1-7 years. The TRIXY Early Childhood Study is a longitudinal study that aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. For this study, only children aged 1 up to and including 5 years were included.

In total, 182 children participated in this study, 87 children with SCT and 95 nonclinical age matched children from the typical population. Ages ranged from 11 months to 5 years and 11 months (see Table 1 for mean ages per karyotype). Of the 87 children with SCT, 60 children received a prenatal diagnosis (i.e., because of (routine) prenatal screening or advanced maternal age). Of the 27 children who received a postnatal diagnosis, 13 received the diagnosis because of a developmental delay, 12 because of physical and/or growth problems, and 2 because of medical concerns.

Table 1. Mean ages per karyotype

	XXY	XXX	XYY	XY	XX
N	40	28	19	40	55
Mean age in months (SD)	33.48 (17.05)	45.89 (18.74)	37.47 (19.87)	42.28 (18.32)	42.38 (18.86)

Recruitment and assessment took place on two sites: The Trisomy of the X and Y chromosomes (TRIXY) Expert Center the Netherlands, and the eXtraordinarY Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado in the USA. Children in the SCT group were recruited with the help of clinical genetics departments (from the Netherlands, Colorado, and Belgium), pediatricians, and national advocacy or support groups for individuals with SCT with recruitment flyers and postings on the internet (e.g., TRIXY website and the eXtraordinarY Kids Facebook page). For the SCT group, ascertainment bias was assessed, three subgroups were identified: (1) 'active prospective follow-up', which included families who were actively followed after prenatal diagnosis (50% of the SCT group), (2) 'Information seeking parents', which included families who were actively looking for more information about SCT without having specific concerns about the behavior of their child (31% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development (18% of the SCT group). Nonclinical controls were recruited from the western part of the Netherlands. Schools and day care centers received information brochures that were distributed to parents with children of eligible age. Parents who were interested in participating contacted the researchers.

For all participants, inclusion criteria were Dutch or English-speaking (child and parent). For the SCT group, SCT was defined by trisomy in at least 80% of the cells, which was confirmed in the study by standard karyotyping. Exclusion criteria for all participants included a history of traumatic brain injury, neurological illness, severely impaired hearing or sight, or colorblindness. For ethical reasons, children in the control group were not subjected to genetic screening, as these children were meant to be a representation of the general population. As the prevalence of SCT is approximately 1 in 1000, the risk of having one of more children with SCT in the control group was considered minimal and acceptable.

For all children, background information such as the presence of a second caregiver and marital status and age of the primary caregiver was assessed. Overall, 95.6% of the parents indicated that their child has a second caregiver, with no significant differences between the SCT and the nonclinical control group χ^2 (1, N = 182) = .36, p = .55. Regarding marital status of the primary caregiver, 92.9% indicated that they were (re)married, registered partners, or living with their partner. Of the remaining parents, 4.4% indicated that they were single and never married, 2.2% indicated that they were single and divorced, and 0.5% indicated that they were widowed. The distribution of marital status was similar for children in the SCT and children in the nonclinical control group χ^2 (3, N = 182) = 2.37, p = .50. Finally, the age of the primary caregiver (93% female) ranged from 23-50 years. There was a significant difference between the research groups (p < .001); on average, the primary caregivers of the children in the SCT group were older (M = 38.51, SD = 4.71) than the primary caregivers of the children in the nonclinical control group (M = 35.06, SD = 5.18).

Instruments

Overall Social-Emotional Functioning

Parents completed the age-appropriate version of the Ages-and-stages social-emotional questionnaire (ASQ-SE-2; Squires et al., 2015). The ASQ-SE-2 is a parent-report screening measure of social and emotional development and can be used to assess children aged 1 to 72 months. Different forms are used, depending on the age of the child, with the number of questions ranging from 19 to 33. The items on the ASQ-SE-2 address seven behavioral constructs: (1) Self-regulation, (2) compliance, (3) adaptive functioning, (4) autonomy, (5) affect, (6) social-communication, and (7) interaction. Parents can respond to each item with 'rarely or never', 'sometimes', or 'most of the time'. In addition, parents can indicate if the behavior is a concern for each item. Answers on the seven constructs add up to a total score, with higher scores indicating increased risk for social-emotional deficits or delays.

Behavioral Functioning

Parents were asked to complete the Child Behavior Checklist (CBCL; Achenbach & Ruffle, 2000) for children aged 1-5 years. The CBCL is a standardized measure of behavioral problems and is used to assess competencies and psychopathology. The CBCL contains 100 items, which assess emotional and behavioral problems that occurred in the past six months. Parents can answer each item with one of the following answers: (0) not true, (1) somewhat or sometimes true, (2) very true or often true, with higher scores indicating more problems. Answers on the items yield empirical syndrome scales and DSM-oriented scales. For this study, the DSM-Oriented scales were used, to assess behavioral functioning, since these are based on profiles

more than on individual behavioral items. The DSM-Oriented scales consist of five different profiles: (1) Affective problems (as indication for mood disorders), (2) anxiety problems, (3) pervasive developmental problems (as indication of disorders on the autism spectrum), (4) attention deficit/hyperactivity problems, and (5) oppositional defiant problems. These five scales overlap with the Diagnostic and Statistical Manual of Mental Disorders (4th ed. American Psychiatric Association, 2013)

Procedure

This study was approved by the Ethical Committee of Leiden University Medical Center, the Netherlands, and the Colorado Multiple Institutional Review Board (COMIRB) in Colorado, USA. After providing a description of the study to the parent(s) of the child, written informed consent according to the declaration of Helsinki was obtained. The primary caregiving parent (93% mother) of the child completed both questionnaires, either in Dutch or in English.

Assessment took place at different sites (Colorado USA, the Netherlands, Belgium). Researchers from Leiden University were responsible for project and data-management (i.e., training and supervision of researchers, processing and scoring of data).

Statistical Analyses

Raw Scores versus Standardized Scores

For both measurements, two types of scores were used. First, raw scores were used to compare the children with SCT and the nonclinical controls. As the ASQ-SE-2 has different items depending on age, ASQ raw scores were corrected for the maximum possible score (which depended on the form used). Secondly, normed or cutoff scores were used for risk assessment. For the CBCL standardized T-scores ($M = 50$, $SD = 10$) were used, where $T < 65$ was classified as 'non-clinical', $65<T<70$ as 'borderline', and $T>70$ as 'clinical'. For the ASQ-SE-2, cutoff scores were used (depending on the form used) where children were categorized as 'below risk/below cutoff', 'borderline/monitoring area', or 'at risk/above cutoff'.

Age Groups

Participants were divided into age groups; resulting in three groups (1) aged 11-23 months (labeled as the 1-year-old group or early toddlerhood; $N_{SCT} = 31$, $N_{controls} = 29$), (2) 24-47 months (labeled as the 2–3-year-old group, or late toddlerhood; $N_{SCT} = 27$, $N_{controls} = 23$), and (3) 48-71 months (labeled as the 4–5-year-old group, or preschool-age; $N_{SCT} = 29$, $N_{controls} = 43$). With a one-way analysis of variance (ANOVA), we tested if there were age differences between the SCT and nonclinical control group within each age group. There were no statistically significant differences, $F(1,180) = 1.83$, $p = .178$.

Analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 25. Level of significance was set at $p \leq .05$, two-tailed. Multivariate analysis of variance (MANOVA) was used to test for differences, with the ASQ-SE-2 and the CBCL-DSM Scales (affective, anxiety, pervasive developmental, attention deficit, oppositional defiant) as dependent variables and research group and age groups as independent variables. When unequal variance-covariance was indicated (i.e., Box's M test $p < .05$), Pillai's trace was used to assess the multivariate effect. Significant multivariate effects were then further analyzed with

univariate ANOVAs and simple effect analyses to determine the locus of the statistically significant multivariate effect. Risk assessment was done with cross-tabulation analysis. Post hoc analyses were used to identify significant group effects. Effect sizes were calculated with Cohen's *d* when applicable.

Results

First, we addressed the question what the behavioral profile of children ages 1-5 with SCT looks like. As different behaviors are expected at different ages, the main focus is on differences within age groups (SCT versus nonclinical controls) and between age groups within the SCT group (to assess developmental stability). Lastly, the behavioral profile of boys (with versus without SCT) and of girls (with versus without SCT) aged 1-5 years was compared, and the effect of time of diagnosis and ascertainment was assessed.

Social-Emotional Functioning and Behavioral Difficulties: SCT versus Nonclinical Controls

There was a significant effect of research group on behavioral phenotype (social-emotional functioning and behavioral difficulties), Pillai's trace = .262, $F(6,175) = 10.37$, $p < .001$, partial $\eta^2 = .262$.

Univariate ANOVAs for the social-emotional scale and the five DSM scales indicated that on average, children with SCT showed more problems in overall social-emotional functioning, and more behavioral symptoms of affective and pervasive developmental problems compared to the nonclinical control group. Cohen's *d* effect sizes (see Table 2) indicate moderate to high clinical significance. For the anxiety, attention deficit and oppositional defiant scales, there was no significant difference in the behavioral symptoms.

Table 2. Behavioral differences SCT versus control

	SCT ***N* = 87**	**Controls** ***N* = 95**	***p***	**Cohen's *d***
ASQ-SE-2[a]	Mean (SD)	Mean (SD)		
Social-Emotional	11.48 (10.14)	5.37 (3.79)	< .001	.80
CBCL DSM scales[a]				
Affective	2.72 (2.13)	1.49 (1.49)	< .001	.67
Anxiety	3.33 (3.32)	2.52 (2.30)	.053	.28
Pervasive Developmental	5.05 (4.23)	2.79 (2.23)	< .001	.67
Attention Deficit	4.57 (2.72)	4.05 (2.50)	.179	.20
Oppositional Defiant	3.53 (3.08)	3.59 (2.43)	.882	.02

[a] Higher scores denote more problems.

In addition to average outcomes, we were also interested how many of the children in each group scored around or above clinical cutoff. Cross-tabulation analysis was used for risk assessment; i.e., how many of the children in each group scored in the nonclinical, borderline, and clinical range. As the CBCL provides normed scores for children aged 18 months and above, children younger than 18 months were excluded from the cross-tabulation analyses with CBCL DSM scores. All children were included in the analysis with ASQ social-emotional scores. Numbers were divided by the total number of participants in each group and shown in Table 3 as percentages per group. Pearson Chi-Square indicates significant group differences for overall social-emotional functioning, and for affective problems, anxiety problems, and pervasive developmental problems, indicating differences in distribution between groups (see Figure 1 for a visual representation).

Table 3. Percentages of children at risk for behavioral problems

	Research Group	Risk Assessment			χ^2 significance
ASQ-SE-2[a]		**Below Risk**	**Monitoring Area**	**At Risk**	
Social-Emotional Functioning	SCT	59.8%	18.4%	21.8%	<.001
	Control	95.8%	2.1%	2.1%	
CBCL DSM Scales[a]		**Nonclinical T<65**	**Borderline 65<T<70**	**Clinical T>70**	
Affective	SCT	88.4%	4.3%	7.2%	.018
	Control	98.8%	1.2%	0%	
Anxiety	SCT	84.1%	1.4%	14.5%	.019
	Control	95.3%	2.4%	2.4%	
Pervasive Developmental	SCT	62.3%	14.5%	23.2%	<.001
	Control	94.1%	3.5%	2.4%	
Attention Deficit	SCT	95.7%	0%	4.3%	.316
	Control	97.6%	1.2%	1.2%	
Oppositional Defiant	SCT	85.5%	7.2%	7.2%	.189
	Control	94.1%	2.4%	3.5%	

[a] Higher scores denote more problems

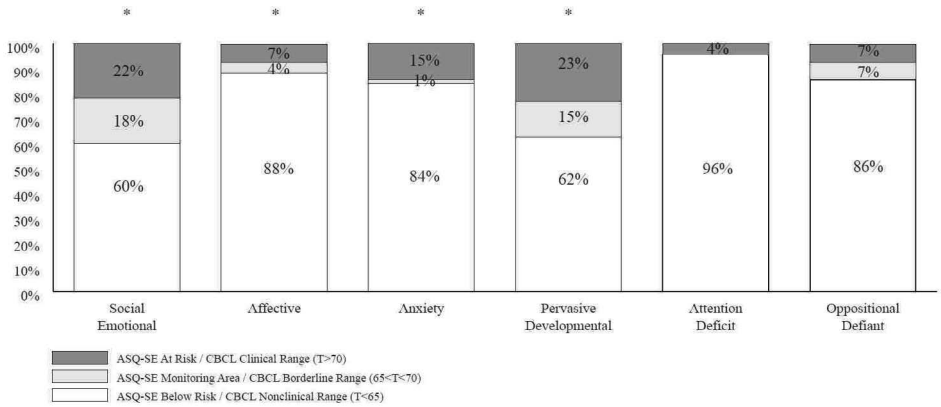

Figure 1. Proportion of children with SCT with ASQ-SE and CBCL DSM cut-off scores in the below risk/nonclinical, monitoring/borderline, and at risk/clinical ranges. Note: * = Distribution significantly different compared to nonclinical controls (p < .05); N_{sct} DSM scales = 69, N_{sct} ASQ-SE = 87

Social-Emotional Functioning and Behavioral Difficulties Across Ages

Within each age group, differences in the behavioral outcomes between the SCT and nonclinical control group were analyzed with three separate MANOVAs. Descriptive statistics for all MANOVAs can be found in Table 4.

1-year-old Children: Early Toddlerhood

There was a significant effect of research group on behavioral phenotype (social-emotional functioning and behavioral difficulties), Pillai's trace = .292, $F(6,53) = 3.64$, $p = .004$, partial $\eta^2 = .292$.Univariate ANOVAs for the social-emotional scale and the five DSM scales indicated significant differences for oppositional defiant behavior and overall social-emotional functioning. On average, children with SCT showed more problems in overall social-emotional functioning than nonclinical controls (see Table 4 for descriptives, and Figure 2). Conversely, for oppositional defiant behavior, children with SCT on average showed fewer problems than nonclinical controls. No significant group differences were found for affective problems,

anxiety problems, pervasive developmental problems, and attention deficit problems, indicating that in 1-year-olds, children with SCT showed similar amounts of these behaviors to nonclinical controls.

2-3 year-old Children: Late Toddlerhood

There was a significant effect of research group on behavioral phenotype (social-emotional functioning and behavioral difficulties), Pillai's trace = .369, $F(6,43) = 4.19$, $p = .002$, partial $\eta^2 = .369$. Univariate ANOVAs for the social-emotional scale and the five DSM scales indicated significant differences for overall social-emotional functioning, and for affective and pervasive developmental problems. On average, children with SCT showed more problems in overall social-emotional functioning, and more behavioral symptoms of affective problems and pervasive developmental problems than nonclinical controls (see Table 4 for descriptives, and Figure 2). No significant group differences were found for anxiety problems, attention deficit problems or oppositional defiant problems, indicating that in 2-3 year-olds, children with SCT group showed similar amounts of these behaviors to nonclinical controls.

4-5 year-old Children: Preschool-Age

There was a significant effect of research group on behavioral phenotype (social-emotional functioning and behavioral difficulties), Pillai's trace = .346, $F(6,65) = 5.72$, $p < .001$, partial $\eta^2 = .346$.

Univariate ANOVAs for the social-emotional scale and the five DSM scales indicated significant differences for all scales (see Table 4 for descriptives and Figure 2). On average, children with SCT showed more problems in overall social-emotional functioning and more, behavioral symptoms of affective problems, anxiety problems, and pervasive developmental problems. In addition, children with SCT also showed more behavioral symptoms of attention deficit problems and oppositional defiant problems than nonclinical controls.

Table 4. Behavioral problems across age groups

	1-year-olds			**2-3 year-olds**			**4-5 year-olds**		
	SCT N=31	**Control N=29**		**SCT N=27**	**Control N=23**		**SCT N=30**	**Control N=43**	
ASQ-SE-2[a]	**Mean (SD)**	**Mean (SD)**	***p***	**Mean (SD)**	**Mean (SD)**	***p***	**Mean (SD)**	**Mean (SD)**	***p***
Social-Emotional Functioning	8.74 (4.95)	5.60 (3.09)	.005	11.70 (7.16)	4.87 (3.51)	<.001	14.20 (15.04)	5.49 (4.37)	.001
CBCL DSM scales[a]									
Affective	1.71 (1.37)	1.28 (1.60)	n.s.	2.89 (2.23)	1.26 (1.42)	.004	3.66 (2.29)	1.77 (1.45)	<.001
Anxiety	1.94 (1.79)	2.07 (1.71)	n.s.	3.07 (2.56)	2.26 (1.86)	n.s.	5.07 (4.36)	2.95 (2.77)	.014
Pervasive Developmental	2.19 (2.34)	1.83 (1.71)	n.s.	5.37 (3.33)	2.78 (1.68)	.001	7.79 (4.66)	3.44 (2.58)	<.001
Attention Deficit	3.87 (2.63)	4.17 (2.27)	n.s.	4.22 (2.21)	3.78 (2.35)	n.s.	5.66 (3.02)	4.12 (2.76)	.029
Oppositional Defiant	1.65 (1.89)	3.24 (2.34)	.005	3.85 (2.60)	4.00 (2.11)	n.s.	5.24 (3.46)	3.60 (2.67)	.027

[a] Note: Higher scores denote more problems
Abbreviations: n.s. = not significant

Developmental Stability

To assess whether there is developmental stability or variability of problem behavior, a MANOVA was used to test for significant differences, with the Social-Emotional Scale and the DSM Scales (affective, anxiety, pervasive developmental, attention deficit, oppositional defiant) as dependent variables and research group and age groups as independent variables. Only the outcomes of the research group x age group interaction will be reported.

There was no significant research group x age group interaction effect on behavioral phenotype (social-emotional functioning and behavioral difficulties), Pillai's trace = .111, $F(12,344) = 1.69$, $p = .068$, partial $\eta^2 = .056$. Univariate effects however, showed significant research group x age group interactions for affective problems ($F(2,176) = 3.04$, $p = .050$, partial $\eta^2 = .033$), pervasive developmental problems ($F(2,176) = 7.57$, $p = .001$, partial $\eta^2 = .079$), and oppositional defiant problems ($F(2,176) = 6.38$, $p = .002$, partial $\eta^2 = .068$). Significant effects were further analyzed with simple effect analyses, relevant means can be found in Table 4.

Affective Problems

The statistically significant effect was produced by the 2–3-year-old, and the 4–5-year-old SCT children, who showed significantly more affective problems than the 2–3-year-old, and 4–5-year-old nonclinical controls. Conversely, in the 1-year-old group, both the SCT children and the nonclinical controls showed similar amounts of affective problems. These results collectively indicate that it is possible that – in this cross-sectional sample – the developmental trajectory is different for SCT children and nonclinical controls (see Figure 2).

Pervasive Developmental Problems

The statistically significant effect was produced by the 2–3-year-old, and the 4–5-year-old SCT children, who showed significantly more pervasive developmental problems than the 2–3-year-old, and 4–5-year-old nonclinical controls. Conversely, in the 1-year-old group, both the SCT children and the nonclinical controls showed similar amounts of pervasive developmental problems. These results collectively indicate that possibly – in this cross-sectional sample – the developmental trajectory is different for SCT children and nonclinical controls (see Figure 2).

Oppositional Defiant Problems

The statistically significant effect was produced by the 4–5-year-old SCT children, who showed significantly more oppositional defiant problems than the nonclinical controls. Conversely, in the 1-year-olds, the children with SCT showed significantly fewer oppositional defiant problems than nonclinical controls. Finally, in the 2–3-year-old group, both the SCT children and the nonclinical controls showed similar amounts of oppositional defiant problems. These results collectively indicate that it is possible that – in this cross-sectional sample – the developmental trajectory is different for SCT children and nonclinical controls (see Figure 2).

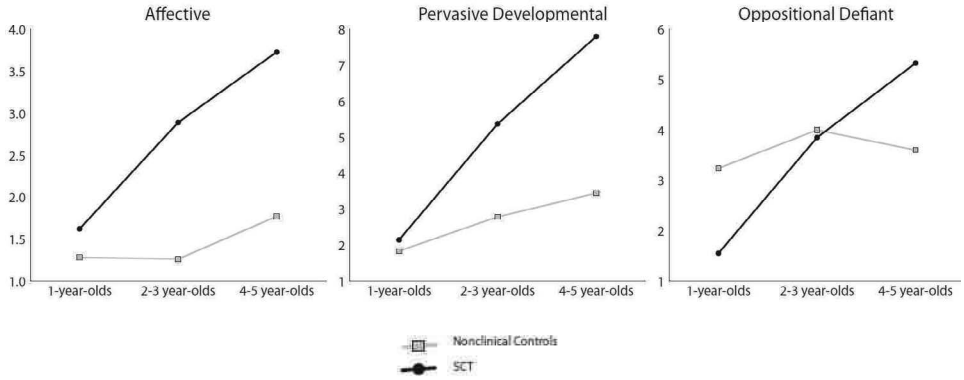

Figure 2. Mean scores for Affective behavior, Pervasive Developmental behavior, and Oppositional Defiant behavior at different ages: SCT versus nonclinical controls

Social-Emotional and Behavioral Differences between Groups: Gender/Karyotype Differences, Time of Diagnosis, and Ascertainment

As we were also interested in the specific behavioral profile of boys and girls, and the individual karyotype group, we compared boys and girls separately (i.e., girls with/without +1X, boys with/without +1X, and boys with/without +1Y). Also, the effect time of diagnosis and the reason for enrollment (i.e., ascertainment) were assessed separately. It should be noted that the factor age was left out of these analyses; results are shown as averages across the whole age range (1-6 years).

Social-Emotional and Behavioral Differences between Gender/Karyotype

Three one-way between-subjects multivariate analyses of variance (MANOVA) were conducted on six dependent variables (CBCL-DSM scales; affective, anxiety, pervasive developmental, attention deficit, oppositional defiant, and the ASQ social-emotional scale). The independent variables were Karyotype (XXX, XX), (XXY, XY), and (XYY, XY).

Table 5. Behavioral differences between groups: Gender differences

	XXX N=29	**XX N=55**		**XXY N=40**	**XY N=40**		**XYY N=19**	**XY N=40**	
	Mean (SD)	**Mean (SD)**	***p***	**Mean (SD)**	**Mean (SD)**	***p***	**Mean (SD)**	**Mean (SD)**	***p***
ASQ-SE-2[a]									
Social-Emotional Functioning	11.00 (9.64)	5.36 (3.40)	<.001	9.56 (6.32)	5.39 (4.32)	.001	16.21 (15.27)	5.39 (4.32)	<.001
CBCL DSM scales[a]									
Affective	2.93 (1.86)	1.49 (1.35)	<.001	2.43 (2.18)	1.50 (1.70)	.037	3.05 (2.39)	1.50 (1.70)	.006
Anxiety	4.89 (4.09)	2.64 (2.45)	.002	2.38 (2.15)	2.35 (2.10)	n.s.	3.05 (3.42)	2.35 (2.10)	n.s.
Pervasive Developmental	6.18 (3.90)	2.71 (2.27)	<.001	3.85 (3.16)	2.90 (2.21)	n.s.	5.89 (5.92)	2.90 (2.21)	.006
Attention Deficit	5.11 (2.90)	4.25 (2.53)	n.s.	3.93 (2.46)	3.78 (2.47)	n.s.	5.16 (2.79)	3.78 (2.47)	n.s.
Oppositional Defiant	4.29 (3.09)	3.51 (2.64)	n.s.	2.85 (3.03)	3.70 (2.15)	n.s.	3.84 (3.01)	3.70 (2.15)	n.s.

[a] Note: Higher scores denote more problems
Abbreviations: n.s. = not significant

There was a significant effect of karyotypes on behavioral phenotype (social-emotional functioning and behavioral difficulties) (XXX Pillai's trace = .345, $F(6,76) = 6.67$, $p < .001$, partial $\eta^2 = .345$; XXY Pillai's trace = .320, $F(6,73) = 5.72$,, $p < .001$, partial $\eta^2 = .320$; XYY Pillai's trace = .351, $F(6,52) = 4.69$, $p = .001$, partial $\eta^2 = .351$). Univariate ANOVAs for the social-emotional scale and the five DSM scales were conducted on each dependent measure separately for each karyotype to determine the locus of the statistically significant multivariate effect. Results are shown in Table 5.

Time of Diagnosis: Prenatal versus Postnatal Diagnosis

A one-way between-subjects multivariate analysis of variance (MANOVA) was conducted on six dependent variables (CBCL-DSM scales; affective, anxiety, pervasive developmental, attention deficit, oppositional defiant, and the ASQ social-emotional scale). The independent variable was time of diagnosis (prenatal, postnatal, controls).

There was a significant effect of time of diagnosis on behavioral phenotype (social-emotional functioning and behavioral difficulties), Pillai's trace = .460, $F(12,350) = 8.70$, $p < .001$, partial $\eta^2 = .230$. Univariate ANOVAs for the social-emotional scale and the five DSM scales indicated significant differences for all scales with the exception of attention deficit problems, which was not significant. Post-hoc analyses were used to determine which group differences were significantly different (see Table 6). For overall social-emotional functioning, children with SCT, regardless of time of diagnosis, showed more problems than controls. For affective problems, pervasive developmental problems and oppositional defiant problems, children who were diagnosed postnatally showed significantly more of these behavioral problems than children with a prenatal diagnosis and controls, with the latter not significantly differing. For anxiety problems, although there was a significant group effect, post-hoc analysis failed to reach significance.

Table 6. Differences in behavioral problems: Time of diagnosis

	Prenatal ***N*** **= 60**	**Postnatal** ***N*** **= 27**	**Controls** ***N*** **= 95**	***p***	**Post-hoc**
ASQ-SE-2[a]	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**		
Social-Emotional Functioning	9.83 (6.20)	15.13 (15.26)	5.37 (3.79)	<.001	C < Pre = Post
CBCL DSM scales[a]					
Affective	2.12 (1.72)	4.07 (2.69)	1.49 (1.49)	< .001	C = Pre < Post
Anxiety	2.78 (2.69)	4.56 (4.21)	2.52 (2.30)	.004	n.s.
Pervasive Developmental	3.78 (3.30)	7.85 (4.75)	2.79 (2.23)	< .001	C = Pre < Post
Attention Deficit	4.33 (2.69)	5.11 (2.75)	4.05 (2.50)	n.s.	n/a
Oppositional Defiant	2.78 (2.62)	5.11 (3.42)	3.59 (2.43)	.001	C = Pre < Post

[a] Note : Higher scores denote more problems

Abbreviations: n.s. = not significant; c = nonclinical controls; pre = prenatal diagnosis of SCT; post = postnatal diagnosis of SCT

Ascertainment Bias

Within the SCT group, we tested for differences on the behavioral outcomes between the three ascertainment groups with MANOVA. There were no significant differences for the behavioral outcomes (see table 7); how children enrolled in the study did not appear to affect the data on behavioral outcomes.

Table 7. Differences in behavioral profiles across ascertainment groups

	Prospective follow-up ***N*** **= 44**	**Information seeking parents** ***N*** **= 27**	**Clinically referred cases** ***N*** **= 16**	***p***
ASQ-SE-2[a]	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
Social-Emotional Functioning	10.70 (10.96)	11.60 (10.38)	13.40 (7.28)	.682
CBCL DSM scales[a]				
Affective	2.36 (2.18)	2.74 (1.87)	3.69 (2.21)	.137
Anxiety	2.98 (2.81)	3.81 (4.04)	3.50 (3.39)	.571
Pervasive Developmental	4.18 (4.33)	5.78 (4.15)	6.19 (3.78)	.173
Attention Deficit	4.61 (2.70)	4.30 (3.06)	4.94 (2.24)	.726
Oppositional Defiant	3.39 (3.32)	3.26 (3.11)	4.38 (2.25)	.507

[a] Note: Higher scores denote more problems

Discussion

This study aimed to describe the early behavioral profile of toddlers and preschoolers with SCT, and more specifically to identify if the presentation of the behavioral phenotype is age-dependent in a large group of children with SCT aged 1-5 years. First, we addressed the question whether behavioral problems could already be found in very young children; between the ages of 1-5 years. Results indicated that children with SCT showed more problems with overall social-emotional functioning, and more behavioral symptoms of affective and pervasive developmental problems than children without SCT. Effect sizes indicated moderate to high clinical significance, indicating that these behaviors are important to monitor during development. When we look at risk assessment, much variability within the SCT group was found, with some children showing no (behavioral) problems, and other children showing (behavioral) problems at a clinical level. Overall, the majority of children with SCT scored within the nonclinical range on the CBCL and ASQ-SE-2 (Table 3). However, there were significantly more children in the SCT group than the control group in the borderline or clinical range for overall social-emotional functioning, and for affective, anxiety, and pervasive developmental behavioral problems, with overall social-emotional functioning and pervasive developmental behaviors seeming to be affected the most. These findings are in concordance with results of similar studies evaluating categorical results of behavioral findings such as Ross et al. (2012), and Tartaglia, Cordeiro, et al. (2010). In sum, these results show that in some children with SCT differences in overall social-emotional functioning can be identified even at a very young age (as early as in 1-year-old children) and that when problems are present, they are highest in the domains of affective and pervasive developmental behaviors.

Key to our research question, we further explored the question whether differences in behavioral problems between children with and without SCT were age dependent. Already in 1-year-olds, there were significant differences between the SCT and control group in overall social-emotional functioning; children with SCT showed more difficulties with overall social-emotional functioning than the nonclinical controls. Oppositional defiant problems, however, were less frequent in the SCT group compared to the control group. In the 2–3-year-old group, the children with SCT also showed more problems in overall social-emotional functioning, in addition to more affective and pervasive developmental problems. Finally, in the 4–5-year-olds, the children with SCT showed more problems across all domains. Taken together, these results show that already in toddlerhood, children with SCT are at risk for suboptimal behavioral development, and this risk increases and expands across behavioral domains as children get

older. From a developmental perspective, it is possible that a subset of challenging behaviors will not emerge until later in development, depending on brain maturation. For example, in our study, only the 4–5-year-olds with SCT showed increased levels of ADHD symptoms, which fits with ADHD typically being diagnosed later in development (i.e., around 7-9 years;), when attentional expectations increase. These findings deserve additional study with a longitudinal study design, and with consideration of other factors that may contribute to behavioral differences, such as cognitive or language skills.

In addition, we addressed the question whether there was developmental stability or variability of problem behavior; i.e., is the developmental path – within this cross-sectional sample – the same in the SCT group as it is in the control group. Results indicated that there was developmental variability for affective behavior, pervasive developmental behavior, and oppositional defiant behavior. Although children with SCT did not differ from nonclinical controls (or in the case of oppositional defiant behavior, showed even fewer problems) in early toddlerhood, children with SCT showed more problem behaviors in late toddlerhood and preschool age. While this is a cross-sectional sample, these findings suggest that the developmental path may be different for controls and children with SCT, and that the impact of behavior problems between children with and without SCT increases as children get older. It should also be noted that for example overall social-emotional functioning did not show this developmental variability, but a more stable development, which fits with our other findings that children with SCT scored differently than controls on all ages; problems with overall social-emotional functioning are persistent over time.

When exploring differences of each karyotype compared to sex-matched controls, results showed that social-emotional and affective domains were higher across all groups. However, anxiety symptoms were more significant in only the XXX group, and pervasive developmental problems only in XXX and XYY. This pattern is interesting and consistent with previous studies evaluating ASD symptoms in older male children with SCT, where males with XYY have been shown to have higher risk for pervasive developmental and autism symptoms compared to XXY (Cordeiro et al., 2012; Ross et al., 2012; Tartaglia et al., 2017). Further, anxiety symptoms and anxiety disorders are recognized as risks in XXX in later childhood and adulthood (Freilinger et al., 2018; van Rijn & Swaab, 2015; Wigby et al., 2016), and these findings suggest symptoms of anxiety may be detected in some very young girls with XXX, which gives promise for early detection and intervention opportunities. Pervasive developmental and autism symptoms have also been identified in other older cohorts with XXX (Bishop et al., 2011; van Rijn et al., 2014), and further study of the prevalence and profile of clinical autism diagnosed is needed for girls with XXX.

When we look at time of diagnosis, it appears that even children with a prenatal diagnosis on average display more difficulties with overall social-emotional functioning than controls; indicating that difficulties with social-emotional functioning can be very persistent. In addition, children with a postnatal diagnosis, often show more behavior problems compared to both controls and prenatally diagnosed children with SCT. This has been shown consistently in other studies (Bardsley et al., 2013; Bishop et al., 2011; Samango-Sprouse et al., 2018), and is very important in counseling families with a prenatal diagnosis. This finding is not surprising,

as postnatal diagnosis is often made because of behavioral and/or physical problems. In addition, it is possible that parents who receive the diagnosis before birth are more aware of the possibilities of (behavioral) outcomes, and for that reason possibly already participate in interventions and preventive support, such as psychoeducation or behavioral interventions at a young age. These outcomes stress the need for early identification and monitoring, and for more comprehensive evaluation of the longitudinal behavioral profiles in a prenatally identified cohort.

Lastly, we looked at ascertainment bias, and found no significant differences between the prospective follow-up group, information seeking parents group, or clinically referred cases group. It is important to note however, that bias within the research sample will always be present. Although it is expected that more individuals will be diagnosed with the introduction of less invasive methods during pregnancy (Samango-Sprouse et al., 2017), two decades ago, only around 25% of individuals with SCT was diagnosed (Abramsky & Chapple, 1997). As non-invasive prenatal screening is not part of routine screening in all countries, the percentage of individuals who will be diagnosed is variable, and results of research will not be generalizable to all individuals with SCT. However, it is possible to generalize our results to children *who are diagnosed* with SCT.

This study has both strengths and limitations. One of the limitations of this study is its design, with a cross-sectional rather than a longitudinal perspective. It is important that future studies will follow children over time, to monitor the behavioral pattern across ages. It should be noted however, that (to our knowledge) this is one of the first studies to research the behavioral profile of very young children with SCT. In addition, with our relatively large sample size, we were able to look for behavioral differences at specific ages (i.e., early toddlerhood, late toddlerhood, preschool age); our results highlight the importance of early identification of children at risk and show that already when a child is one-year-old problem behaviors, especially with overall social-emotional functioning, can occur. Future research could focus on neurocognitive and environmental factors (e.g., SES and services received) that could serve as risk- or protective factors in the development of behavior, as there is a complex relation between genetics, environmental factors and neuro(behavioral) development (Karmiloff-Smith, 2009).

Social-emotional and behavioral problems have been negatively associated with a child's daily functioning. Social competence, school performance, and peer acceptance, for example, can be affected because a child experiences behavioral problems (de Lijster et al., 2019). The presence of behavioral problems during early childhood could be predictive of later psychopathology and severity of behavioral problems at a later age (Goodwin et al., 2004; Ormel et al., 2015; Roza et al., 2003). Even though both the CBCL and the ASQ-SE-2 are screening instruments rather than diagnostic evaluations, results on these screeners clearly demonstrate higher risks for psychopathology for some children with SCT, and the need for early monitoring and implementation of intervention, especially in the domain of social-emotional functioning.

In conclusion, our findings give some important implications for clinical care. First of all, with the broad behavioral phenotype, it is important to include behavioral screening in

routine clinical care for children with SCT, and to monitor the developmental trajectory. Difficulties with social-emotional development seem most prominent, as there is an increased risk already when children are one year old, and elevated scores were persistent across the full 1–5-year age range, regardless of time of diagnosis, and across all three karyotypes. While each child with SCT is different, our results suggest a pattern of affective and pervasive developmental problems emerging in the late-toddler stage, and finally anxiety, attention deficits, and oppositional defiant problems emerging during the preschool years. It is important to monitor the behavioral development closely, with a focus on these specific domains on specific ages, so interventions and preventive support can be administered as early as possible, to optimize outcomes. Routine screenings should be done from an early age onwards, as behaviors can already be clinically relevant from a very young age and without early assessment, opportunities for early intervention could be missed. In addition, it is important that parents who receive the diagnosis are aware of the wide variability of outcomes, and receive psychoeducation on the possible behavioral problems, in particular affective problems, pervasive developmental problems, and social-emotional development, as our results show that these difficulties already arise at a very young age, and problems possibly could intensify over time. Knowledge about these early neurobehavioral risks should ideally fuel implementation of early interventions and psychoeducation, optimizing outcomes of children with SCT.

References

Abramsky, L., & Chapple, J. (1997). 47,XXY (Klinefelter syndrome) and 47,XYY: Estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenatal Diagnosis*, *17*(4), 363-368. https://doi.org/10.1002/(sici)1097-0223(199704)17:4%3C363::aid-pd79%3E3.0.co;2-o

Achenbach, T., & Ruffle, T. (2000). The child behavior checklist and related forms for assessing behavioral/emotional problems and competencies. *Pediatrics in Review*, *21*, 265-271. https://doi.org/10.1542/pir.21-8-265

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.) [Text Revision]. American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596

Andersen, S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews*, *27*, 3-18. https://doi.org/doi:10.1016/S0149-7634(03)00005-8

Bardsley, M. Z., Kowal, K., Levy, C., Gosek, A., Ayari, N., Tartaglia, N., . . . Ross, J. L. (2013). 47,XYY syndrome: Clinical phenotype and timing of ascertainment. *The Journal of Pediatrics*, *163*(4), 1085-1094. https://doi.org/10.1016/j.jpeds.2013.05.037

Bishop, D. V. M., Jacobs, P. A., Lachlan, K., Wellesley, D., Barnicoat, A., Boyd, P. A., . . . Scerif, G. (2011). Autism, language and communication in children with sex chromosome trisomies. *Archives of Disease in Childhood*, *96*(10), 954-959. https://doi.org/10.1136/adc.2009.179747

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevelance of Klinefelter syndrome: A national registry study. *The Journal of Clinical Endocrinology & Metabolism*, *88*(2), 622-626. https://doi.org/10.1210/jc.2002-021491

Cordeiro, L., Tartaglia, N., Roeltgen, D., & Ross, J. (2012). Social deficits in male children and adolescents with sex chromosome aneuploidy: A comparison of XXY, XYY, and XXYY syndromes. *Research in Developmental Disabilities*, *33*(4), 1254-1263. https://doi.org/10.1016/j.ridd.2012.02.013

de Lijster, J. M., van den Dries, M. A., van der Ende, J., Utens, E., Jaddoe, V. W., Dieleman, G. C., . . . Legerstee, J. S. (2019). Developmental trajectories of anxiety and depression symptoms from early to middle childhood: A population-based cohort study in the Netherlands. *Journal of Abnormal Child Psychology*, *47*(11), 1785-1798. https://doi.org/10.1007/s10802-019-00550-5

Freilinger, P., Kliegel, D., Hanig, S., Oehl-Jaschkowitz, B., Henn, W., & Meyer, J. (2018). Behavioral and psychological features in girls and women with triple-X syndrome. *American Journal of Medical Genetics Part A*, *176*(11), 2284-2291. https://doi.org/10.1002/ajmg.a.40477

Goodwin, R. D., Fergusson, D. M., & Horwood, L. J. (2004). Early anxious/withdrawn behaviours predict later internalising disorders. *Journal of Child Psychology and Psychiatry*, *45*(4), 874-883. https://doi.org/10.1111/j.1469-7610.2004.00279.x

Groth, K. A., Skakkebaek, A., Høst, C., Gravholt, C. H., & Bojesen, A. (2013). Klinefelter syndrome - a clinical update. *The Journal of Clinical Endocrinology & Metabolism*, *98*(1), 20-30. https://doi.org/10.1210/jc.2012-2382

Hong, D. S., & Reiss, A. L. (2014). Cognitive and neurological aspects of sex chromosome aneuploidies. *The Lancet Neurology*, *13*(3), 306-318. https://doi.org/10.1016/s1474-4422(13)70302-8

Karmiloff-Smith, A. (2009). Nativism versus neuroconstructivism: Rethinking the study of developmental disorders. *Developmental Psychology*, *45*(1), 56-63. https://doi.org/10.1037/a0014506

Kessler, R. C., Amminger, G. P., Aguilar-Gaxiola, S., Alonso, J., Lee, S., & Üstün, T. B. (2007). Age of onset of mental disorders: A review of recent literature. *Current Opinion in Psychiatry*, *20*(4), 359-364. https://doi.org/10.1097/YCO.0b013e32816ebc8c

Morris, J. K., Alberman, E., Scott, C., & Jacobs, P. (2008). Is the prevalence of Klinefelter syndrome increasing? *European Journal of Human Genetics*, *16*, 163-170. https://doi.org/10.1038/sj.ejhg.5201956

Ormel, J., Raven, D., van Oort, F., Hartman, C. A., Reijneveld, S. A., Veenstra, R., . . . Oldehinkel, A. J. (2015). Mental health in Dutch adolescents: A TRAILS report on prevalence, severity, age of onset, continuity and co-morbidity of DSM disorders. *Psychological Medicine*, *45*(2), 345-360. https://doi.org/10.1017/S0033291714001469

Ross, J. L., Roeltgen, D. P., Kushner, H., Zinn, A. R., Reiss, A., Bardsley, M. Z., . . . Tartaglia, N. (2012). Behavioral and social phenotypes in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Pediatrics*, *129*(4), 769-778. https://doi.org/10.1542/peds.2011-0719

Roza, S. J., Hofstra, M. B., van der Ende, J., & Verhulst, F. C. (2003). Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: A 14-year follow-up during childhood, adolescence, and young adulthood. *American Journal of Psychiatry*, *160*, 2116-2121. https://doi.org/10.1176/appi.ajp.160.12.2116

Samango-Sprouse, C., Stapleton, E., Chea, S., Lawson, P., Sadeghin, T., Cappello, C., . . . Van Rijn, S. (2018). International investigation of neurocognitive and behavioral phenotype in 47,XXY (Klinefelter syndrome): Predicting individual differences. *American Journal of Medical Genetics Part A*, *176*(4), 877-885. https://doi.org/10.1002/ajmg.a.38621

Samango-Sprouse, C. A., Keen, C., Sadeghin, T., & Gropman, A. L. (2017). The benefits and limitations of cell-free DNA screening for 47,XXY (Klinefelter syndrome). *Prenatal Diagnosis*, *37*(5), 497-501. https://doi.org/10.1002/pd.5044

Samango-Sprouse, C. A., Stapleton, E., Sadeghin, T., & Gropman, A. L. (2013). Is it all the X: Familial learning dysfunction and the impact of behavioral aspects of the phenotypic presentation of XXY? *American Journal of Medical Genetics Part C (Seminars in Medical Genetics)*, *163*(C), 27-34. https://doi.org/10.1002/ajmc.31353

Squires, J., Bricker, D., & Twombly, E. (2015). *Ages & stages questionnaires®: Social-emotional, second edition (ASQ®:SE-2): A parent-completed child monitoring system for social-emotional behaviors*. Paul H. Brookes Publishing Co., Inc.

Tartaglia, N. R., Cordeiro, L., Howell, S., Wilson, R., & Janusz, J. (2010). The spectrum of the behavioral phenotype in boys and adolescents 47,XXY (Klinefelter Syndrome). *Pediatric Endocrinology Reviews*, *8*, 151-159.

Tartaglia, N. R., Howell, S., Sutherland, A., Wilson, R., & Wilson, L. (2010). A review of trisomy X (47,XXX). *Orphanet Journal of Rare Diseases*, *5*, 8. https://doi.org/10.1186/1750-1172-5-8

Tartaglia, N. R., Wilson, R., Miller, J. S., Rafalko, J., Cordeiro, L., Davis, S., . . . Ross, J. (2017). Autism spectrum disorder in males with sex chromosome aneuploidy: XXY/Klinefelter syndrome, XYY, and XXYY. *Journal of Developmental and Behavioral Pediatrics*, *38*(3), 197-207. https://doi.org/10.1097/dbp.0000000000000429

Urbanus, E., Van Rijn, S., & Swaab, H. (2019). A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention. *Clinical Genetics*, *97*(1), 156-167. https://doi.org/10.1111/cge.13586

Van Rijn, S. (2019). A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47,XXX, 47,XYY). *Current Opinion in Psychiatry*, *32*(2), 79-84. https://doi.org/10.1097/yco.0000000000000471

Van Rijn, S., Stockmann, L., Borghgraef, M., Bruining, H., van Ravenswaaij-Arts, C., Govaerts, L., . . . Swaab, H. (2014). The social behavioral phenotype in boys and girls with an extra X chromosome (Klinefelter syndrome and Trisomy X): A comparison with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *44*(2), 310-320. https://doi.org/10.1007/s10803-013-1860-5

Van Rijn, S., & Swaab, H. (2015). Executive dysfunction and the relation with behavioral problems in children with 47,XXY and 47,XXX. *Genes Brain and Behavior*, *14*(2), 200-208. https://doi.org/10.1111/gbb.12203

Verri, A., Cremante, A., Clerici, F., Destefani, V., & Radicioni, A. (2010). Klinefelter's syndrome and psychoneurologic function. *Molecular Human Reproduction*, *16*(6), 425-433. https://doi.org/10.1093/molehr/gaq018

Wigby, K., D'Epagnier, C., Howell, S., Reicks, A., Wilson, R., Cordeiro, L., & Tartaglia, N. (2016). Expanding the phenotype of Triple X syndrome: A comparison of prenatal versus postnatal diagnosis. *American Journal of Medical Genetics Part A*, *170*(11), 2870-2881. https://doi.org/10.1002/ajmg.a.37688

Chapter 3

A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention

Published as:

Urbanus, E., Van Rijn, S., & Swaab, H. (2019). A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention. *Clinical Genetics, 97*(1), 156-167. https://doi.org/10.1111/cge.13586

Abstract

Sex chromosome trisomies (SCT) are among the most common chromosomal duplications in humans. Due to recent technological advances in non-invasive screening, SCT can already be detected during pregnancy. This calls for more knowledge about the development of (young) children with SCT. This review focused on neurocognitive functioning of children with SCT between 0-18 years, on domains of global intellectual functioning, language, executive functioning, and social cognition, in order to identify targets that could benefit from early treatment.

Online databases were used to identify peer-reviewed scientific articles using specific search terms. In total 18 studies were included. When applicable, effect sizes were calculated to indicate clinical significance.

Results of the reviewed studies show that although traditionally, the focus has been on language and IQ in this population, recent studies suggest that executive functioning and social cognition may also be significantly affected already in childhood.

These findings suggest we should extend neuropsychological screening of children diagnosed with SCT, to also include executive functioning and social cognition. Knowledge about these neurocognitive risks is important to improve clinical care and help identify targets for early support and intervention programs to accommodate for the needs of individuals with SCT.

Introduction

Chromosome trisomies are genetic variations caused by a spontaneous error during early cell division (Leggett et al., 2010). Sex chromosome trisomies (SCT), trisomies involving the X or Y chromosomes, are among the most common chromosomal duplications in humans (Hong & Reiss, 2014), with an estimated prevalence ranging from 1- 650 to 1-1000 live births (Bojesen et al., 2003; Groth et al., 2013; Morris et al., 2008). SCT can lead to a 47,XXY (Klinefelter syndrome) or 47,XYY (XYY syndrome) karyotype in males, and a 47,XXX (Trisomy X syndrome) karyotype in females.

Although SCT are relatively common genetic variations, they are also one of the most frequently underdiagnosed chromosomal conditions; up to 75 percent of individuals with SCT are never diagnosed (Abramsky & Chapple, 1997). This high percentage may be explained by several factors. First, physical characteristics are relatively subtle (Lenroot et al., 2014; Otter et al., 2010). Secondly, individuals may be treated for symptoms without knowledge of the underlying genetic condition. Finally, cognitive as well as behavioral symptoms are variable (Giltay & Maiburg, 2010; Tartaglia et al., 2010), ranging from severe impairments in some individuals, with other individuals functioning on an average or above average level. The subtle physical characteristics, and the variability of symptoms often does not prompt to genetic testing. There are certain moments in life when the developing brain is especially sensitive to environmental influences regarding the development of specific neurocognitive functions (Andersen, 2003). It is possible that when the genetic diagnosis is not made or delayed, the so called 'window of opportunity' to explicitly support specific developmental stages passes, which could result in more severe cognitive and/or behavioral difficulties (Wigby et al., 2016).

Focusing on the neurocognitive underpinnings of behavior rather than behavioral symptoms itself is important as behavioral problems may arise as a consequence of different information processing deficits. Also, cognitive deficits may serve as early predictors of behavioral problems in later life and may function as markers for children at risk for neurodevelopmental problems.

Over the last decade, the technology to detect genetic variations in unborn children has advanced significantly; one advantage being that they can be non-invasive, for example by screening maternal blood. These advanced technological developments and the increased possibility to detect SCT during the pregnancy could lead to more individuals being diagnosed on the genetic, instead of the behavioral level (Samango-Sprouse et al., 2017). This calls for more knowledge about the development of (young) children with SCT, so children can get the appropriate support as early as possible when needed. The identification of a profile of neurocognitive risks, and knowledge about the mechanisms underlying these risks, could help improve early screening for neurobehavioral problems in young children with SCT and help identify targets for early, tailored support and intervention programs, which in turn could hopefully optimize outcomes in later life. Although some of these neurocognitive mechanisms are still 'under construction' in early childhood, and for that reason are more apparent in late childhood or adolescence, precursors of some of these mechanisms can already be measured in early childhood.

Through a narrative review of the literature, we evaluated evidence for cognitive impairments on the domains of global intellectual functioning (GIF), language development, executive functioning, and social cognition in children with SCT. Earlier reviews have focused on the development of individuals with SCT over the lifespan, primarily during adolescence and adulthood. In contrast, in this review, neurocognitive functioning of children with SCT was reviewed, with a focus on early development. As the domains of GIF, language development, social cognition, and executive functioning are vulnerable domains based on studies in adolescents and adults and may be key factors that could drive the emotional and behavioral problems that can be found in individuals with SCT (Van Rijn, 2018), it is important to monitor possible developmental risk in these domains already early in life. For that reason, our first aim was to review to what degree impairments in areas of GIF, language development, social cognition, and executive functioning have been studied in children with SCT and identify possible gaps in research that future research should focus on. Secondly, in addition to identifying the type of impairments, we also aimed to determine the degree of impairment, to establish clinical significance and identify risk-factors that should be closely monitored from early development onwards or that should be included in standard clinical neuropsychological screening to identify potential targets for support and intervention. Knowledge about the functioning of children with SCT in these domains is important to be able to identify children who are at risk for lowered adaptive functioning, academic challenges, and psychopathology, and whom thus may be in need of close monitoring and early support or intervention.

Method

Search Strategy

A structured approach was used to identify and review articles. The online database Web of Knowledge was used to identify eligible peer-reviewed scientific articles that were published before July 1, 2018. An overview of the used search terms can be found in Figure 1. The Web of Knowledge categories filter was used to include publications in the following categories: Behavior sciences, education, genetics heredity, language and linguistics, neurosciences, pediatrics, psychiatry, and psychology (clinical, developmental, multidisciplinary). Using the same search strategy, the online database PubMed was consulted, but no additional relevant articles were identified. Finally, reference lists from identified papers were consulted to trace additional papers.

Study Selection

After removing duplicates using the EndNote automatic duplicate removal function, the retrieved articles were scanned for relevance by author 1. Titles and abstracts were assessed by authors 1 and 2 before assessing full texts of studies and discrepancies were resolved via consensus. The inclusion criteria specified that to be eligible for the review (1) Participants in the studies were aged between 0-18 years, or when the study included a broader age range, the effect of age was assessed, (2) Studies were published in international peer-reviewed journals and available as a full-text article written in English, (3) Studies included ≥15 participants, (4) The main focus of the study was on global intellectual functioning, language development, social cognition, or executive functioning. In addition, studies were included regardless of recruitment strategy, including newborn screening studies, as well as studies that included

prenatally diagnosed participants, and postnatal follow-up studies. Ascertainment bias plays a role in much of the literature on SCT. By including studies regardless of recruitment strategy (and thus clinical ascertainment) we aimed to describe as much of the variability on the reviewed domains, even though these outcomes may not be fully representative for the entire SCT population, this means that clinical ascertainment is also part of this review. Table 1 gives an overview of the sample ascertainment of the included studies. Also, studies were included when children with SCT were compared to a (matched)-control group, or when validated instruments were used to compare children with SCT with a normed reference group, an overview of study design of the included studies can be found in Table 1. Finally, studies were included regardless of used instrument type, including both parent report and performance-based tests.

In total, 18 publications met our criteria. For each publication, participant characteristics, study design, and results were summarized in a spreadsheet, which were the basis for the tables in this manuscript. As this is a narrative review, a formal meta-analysis or methodological appraisal was not conducted. However, to indicate the clinical significance of the outcomes reported in the included studies, effect sizes were calculated when applicable.

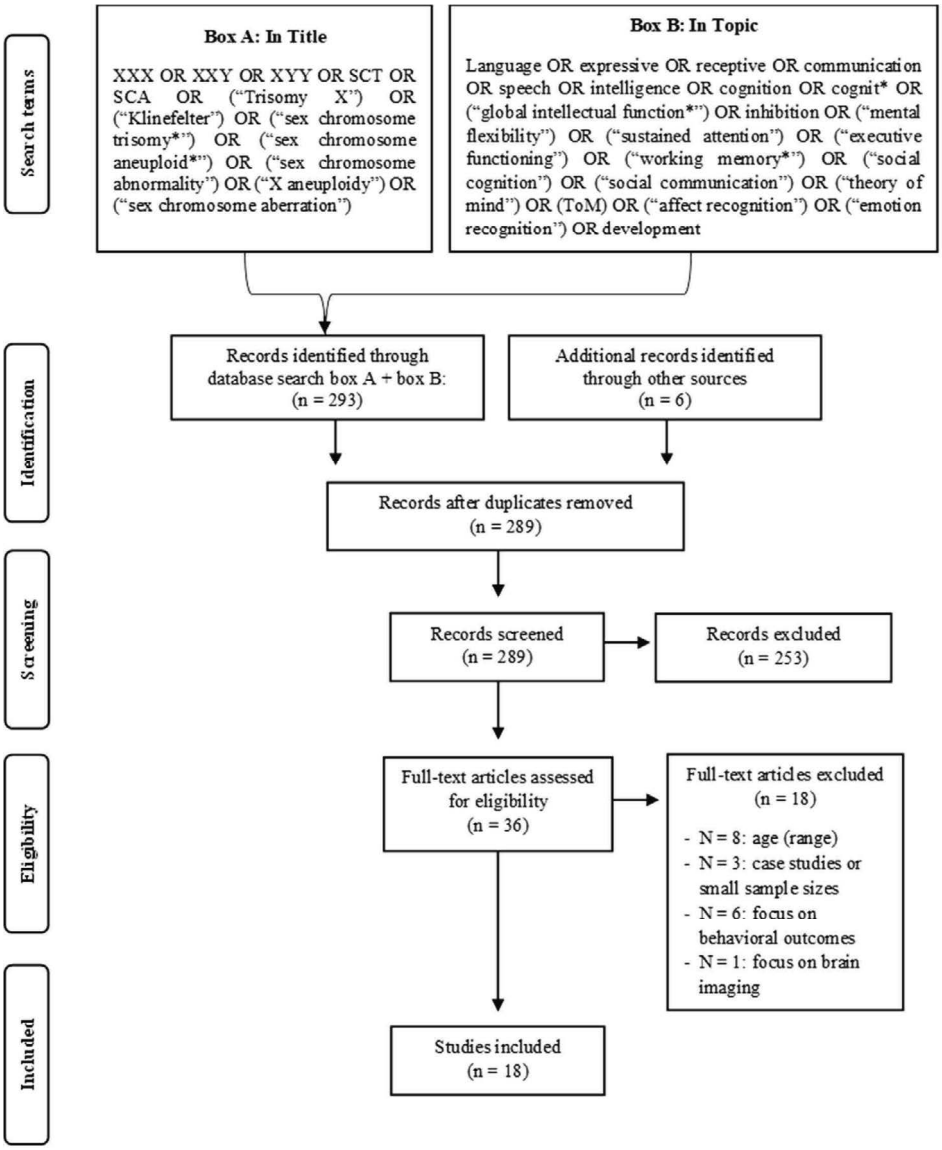

Figure 1. PRISMA flow chart of search strategy and included studies

Table 1. Ascertainment and study design of included studies

Authors	Included Karyotypes	Prenatal diagnosed (%)	Study Design
Ross et al., 2008	XXY	60	Cross-sectional, comparison with normed reference group
Ross et al., 2009	XXY	55	Cross-sectional, comparison with age-matched controls
	XYY	29	
Cordeiro et al., 2012	XXY	56	Cross-sectional, comparison with normed reference group
	XYY	33	
Bruining et al., 2009	XXY	51	Cross-sectional, comparison with normed reference group
Ratcliffe, 1999	XXY	100	Cross-sectional, comparison with controls and siblings
	XYY	95	Cross-sectional, comparison with social class matched controls
	XXX	100	Cross-sectional, comparison with female controls and siblings
Rovet et al., 1995; 1996	XXY	100	Cytogenetic survey followed by longitudinal follow-up comparison with sibling controls
Netley, 1986	XXY	N/A[a]	Summary of several cytogenetic surveys with longitudinal follow-up, comparison group differed between groups, including family member, unrelated controls, or a normed reference group
	XXX		
	XYY		
Zampini et al., 2018	XXX/XXY	100	Cross-sectional, comparison with controls
Haka-Ikse et al., 1978	XXY	100	Cytogenetic survey followed by longitudinal follow-up comparison with normed reference group
Bishop et al., 2011	XXX	51	Cross-sectional, comparison with sibling controls
	XXY	100	
	XYY	36	
Lee et al., 2015	XXY/XXX	100	Cross-sectional, comparison with controls matched on chronological age and maternal education level
Van Rijn & Swaab, 2015	XXX/XXY	53	Cross-sectional, comparison with controls
Samango-Sprouse et al., 2018	XXY (NL)	55	Cross-sectional, comparison with normed reference group
	XXY (US)	91	
Ross et al., 2015	XYY	35	Cross-sectional, comparison with controls matched on chronological age
Van Rijn et al., 2014a	XXX/XXY	53	Cross-sectional, comparison with controls
Van Rijn et al., 2018	XXY	24	Cross-sectional, comparison with normed reference group
Van Rijn et al., 2014b	XXX/XXY	49	Cross-sectional, comparison with controls

[a] Percentage prenatal diagnosed is not explicitly stated in this summary overview

Results

Global Intellectual Functioning

Eight studies met our inclusion criteria regarding global intellectual functioning (GIF). Main findings of the included studies, in addition to used instruments and studied populations can be found in Table 2.

Ross et al. (2008) studied 47 boys with XXY aged 4-18 years and compared scores to a normed reference group. The four- to nine-year-olds showed relative strengths on the non-verbal reasoning subtests (i.e., matrices, sequential and quantitative reasoning) and on the spatial subtests (i.e., recall of design, pattern constructions), in contrast to subtests on the verbal cluster (i.e., word definitions, similarities). The 10-18-year-olds showed low average scores on the verbal and non-verbal reasoning subtests, whereas they had average scores on the spatial cluster subtests. When comparing the younger and older subgroups, it appeared that the older children performed worse on the matrices subtest and had slightly lower general conceptual ability than the younger boys.

A second study by Ross et al. (2009) included 93 boys with XXY, 21 boys with XYY, and 36 matched control boys, aged 4-18 years. General conceptual ability was lower in the XXY and XYY groups, compared to controls. Overall, performance was similar in XXY and XYY boys, with the exception of nonverbal spatial cognitive abilities, which were better (i.e., not different from controls) in boys with XYY.

A cohort of boys aged 4-18 years was included in the study of Cordeiro et al. (2012). Results of GIF were obtained for 95 boys with XXY and 29 boys with XYY. Results showed a wide range of intellectual abilities, with a total IQ ranging from extremely/very low to very superior/high. There were no significant differences between the XXY and XYY groups; in both groups, VIQ was significantly lower than PIQ.

The wide variability of intellectual abilities was also found in a study by Bruining et al. (2009). Forty-seven boys with XXY aged between 6-19 years participated. Total IQ and PIQ scores ranged from extremely low to superior, whereas VIQ scores ranged from extremely low to high average.

In the Edinburgh cohort, 19 boys with XXY, 19 boys with XYY, and 16 girls with XXX were followed from birth until the ages of 16 to 27. Intelligence was tested between the ages of 6-8 years. The XYY boys scored slightly, but significantly, lower than controls matched on social class and sibling controls, especially in the verbal domains. The XXY boys, as well as the XXX girls, scored significantly lower than controls and siblings in both the verbal and the performance domains, and showed a wide variability in scores (Ratcliffe, 1999).

In the Toronto cohort, boys with XXY were followed from birth until the age of 20 years. Intelligence was measured over time at several age intervals, with the sample size ranging from 21 to 29 participants. Results showed that scores on the performance domain were only lower in boys with XXY when compared to controls at the youngest age interval (i.e., 6-8 years), whereas scores on the verbal domain were lower in boys with XXY at all ages, except

when they were 15-17 years. Boys with XXY had poorer verbal scores compared to performance scores at all ages (Rovet et al., 1995; Rovet et al., 1996).

Netley (1986) summarized results of several longitudinal studies, including data from the Boston, Denver, Edinburgh, Japan, Toronto, and Winnipeg cohorts. In total 73 boys with XXY, 32 girls with XXX, and 28 boys with XYY participated and were compared to normed scores. Results showed that boys with XXY scored lower on the verbal, but not performance domains, whereas girls with XXX scored lower on both the verbal and performance domain, with better performance than verbal scores. Finally, no significant differences in GIF were found in boys with XYY.

Language Development

Five studies met our inclusion criteria regarding language development in children with SCT. Main findings of the included studies, in addition to used instruments and studied populations can be found in Table 3. When applicable, effect sizes were calculated to indicate the clinical significance.

Zampini et al. (2018), studied 15 boys and girls with an extra X chromosome at the age of 24 months. Parents from children with an extra X reported that their child produced significantly less words than parents of control children. In addition, 60% of the children with an extra X were at risk for language impairments. In a semi-structured play session between children and their parent, spontaneous utterances, verbal productions, and gestures of the child were coded and classified. During this play session, children with an extra X showed less verbal utterances, and more simple vocal productions. In addition – possibly to compensate – the extra X group showed more pointing gestures. When comparing the boys and girls in the extra X group, no significant differences were found, indicating that, although less pronounced in girls, the language difficulties could be similar in XXX and XXY.

This early risk for language problems was also found in a study by Haka-Ikse et al. (1978), who studied 25 boys with XXY between the ages of three-to-six years and used the revised Yale Developmental Schedules to assess performance on several domains including language. This study showed that already at preschool age, boys with XXY demonstrate a mild developmental delay in language development; with more than half of the children experiencing problems with language.

Two studies used more extensive language assessments and included measures for expressive language, receptive language, phonological processing, phonemic fluency, semantic fluency, and complex levels of language processing (i.e., semantics, syntax, and pragmatics). The first study found age-appropriate development of expressive and receptive vocabulary, as well as normal verbal fluency development in 47 boys with XXY aged 4-18 years (Ross et al., 2008). More complex levels of language processing, however, were impaired. When comparing four-to-nine-year-olds with 10-to-18-year-olds, it appeared that the older group had significantly more difficulties with these complex levels of language processing. The second study compared boys between the ages of 4-18 years with XXY ($N = 93$), XYY ($N = 21$), and controls matched on age (Ross et al., 2009). Results showed that both boys with XXY and XYY perform significantly worse than controls on measures of expressive and receptive language,

with the XYY boys performing worse than the XXY boys. In addition, phonetic fluency was lower in XXY and XYY boys compared to controls, whereas semantic fluency and phonological processing were unimpaired. Finally, complex levels of language processing were impaired in both boys with XXY and XYY. The authors conclude that although boys with XXY and XYY both experience language difficulties, these difficulties appear to be more severe in boys with XYY.

Bishop et al. (2011) relied solely on parent reports. This study included children between the ages of 4-to-16 years and compared children who were diagnosed prenatally versus children who were diagnosed postnatally. More than half of the children with SCT received language therapy, compared to ten percent of the sibling controls. Rates of language therapy were significantly higher among children who were diagnosed postnatally (68%) than children diagnosed prenatally (44%); and more common in boys with XYY (88%) than boys with XXY (47%) or girls with XXX (41%). Parents reported a similar profile of impairments across the SCT groups; however, impairments appeared to be greater in boys than in girls, and in children with a postnatal diagnosis compared to children with a prenatal diagnosis.

Executive Functioning

Five studies met our inclusion criteria regarding executive functioning (EF) in children with SCT. Main findings of the included studies, in addition to used instruments and studied populations can be found in Table 4. When applicable, effect sizes were calculated to indicate the clinical significance.

One study used parent report to assess difficulties with EF and showed that parents with children aged 5-18 years with an extra X chromosome (N = 30) reported more difficulties than parents with typically developing children on all domains (i.e., inhibition, ability to shift behavior, emotional control, working memory, planning/organizing, initiating behavior, and organization of materials). In addition, a cross-sectional study with the same group of participants showed age-effects in the extra X group; although there appeared to be developmental stability (i.e., difficulties did not differ across the age-groups) on most domains, difficulties on initiating and planning/organizing domains, became more pronounced with increased age (Lee et al., 2015).

Four studies used performance-based tasks to examine processing speed, sustained attention, response inhibition, and inhibitory control. In the first study age-appropriate performance on cognitive inhibition tasks was found in 47 boys with XXY (Ross et al., 2008). When comparing four-to-nine-year-olds with 10-to-18-year-olds, it appeared that younger, but not older boys had difficulties with sustained attention. The second study compared boys with XXY (N = 93) or XYY (N = 21) with age-matched controls between the ages of 4-18 years (Ross et al., 2009). Results showed significantly more difficulties with sustained attention in the XXY group, but not the XYY group. However, both the XXY and the XYY group had increased reaction times and showed more variability during the sustained attention task. On inhibition tasks, the XYY, but not the XXY group displayed significantly more difficulties in both inhibiting a cognitive response, and switching between rules within the task, indicating more problems with mental flexibility in boys with XYY. The third study used both computerized performance-based tasks as well as parent reports to assess EF in 23 boys with

XXY and 17 girls with XXX all aged between 9-18 years (van Rijn & Swaab, 2015). This study found no significant differences between the extra X groups and a group of controls on information processing speed, focused attention, or verbal working memory. However, significant group differences were found on measures of sustained attentional control, inhibition, mental flexibility, visual working memory, and daily life executive functioning (as reported by parents). The results for XXY boys and XXX girls were not significantly different, although processing speed was lower in girls with XXX. Finally, differences between children who were diagnosed prenatally versus children with a postnatal diagnosis were not found. The fourth study used the same computerized tasks as the previous study to measure sustained attentional control, inhibition, and mental flexibility in two groups of boys with XXY from the Netherlands (N = 44) and from the United States (N = 54) (Samango-Sprouse et al., 2018). Developmental risk was calculated as a percentage of children that scored in the significantly impaired range (i.e., Z > 2.0). Results showed that 19-23% experienced significant and clinically relevant difficulties with sustained attention. However, difficulties with attention regulation (i.e., stability of reaction times) occurred in 22% of the USA boys, and 57% of the Dutch boys. The authors note that time of diagnosis was a significant predictor for attention regulation, and that 46% of the Dutch boys received a prenatal diagnosis, compared to 91% of the USA boys. On the inhibition task, 26-28% of the children experienced significant and clinically relevant difficulties, and on the mental flexibility task 35-36% experienced significant and clinically relevant difficulties, demonstrating a developmental risk for several EF.

Social Cognition

Six studies met our inclusion criteria regarding social cognition in children with SCT. Main findings of the included studies, in addition to used instruments and studied populations can be found in Table 5. When applicable, effect sizes were calculated to indicate the clinical significance.

Three studies used parent reports to assess social cognition in children with SCT. The first study included 18 boys with XYY between the age of 4-14 years (Ross et al., 2015). The XYY boys had higher scores than controls, indicating more difficulties with social cognition. A second study included children and adolescents with XXY (N = 102) and XYY (N = 40) aged 4-to-18 years (Cordeiro et al., 2012). Parents of boys with XXY and XYY reported more impairments with social cognition, than parents in the normative sample. Parents of XYY boys also reported more impairments than parents of XXY boys. In addition, parents of the XXY and XYY groups both reported more variability in scores compared to the normative sample, indicating a wide range of social cognitive abilities in boys with SCT. The third study included 60 boys and girls with an extra X chromosome, between the ages of 9-18 years (van Rijn, Stockmann, Borghgraef, et al., 2014). Parents of children with an extra X chromosome reported more difficulties in social cognition compared to parents of typically developing children. No significant differences were found in the reported difficulties between boys and girls with an extra X chromosome, indicating similar impairments in social cognition.

Three studies were identified that used child-assessments to measure social cognition skills, such as theory of mind (ToM) and (facial) emotion recognition. The first study involved 70 boys and men with XXY, and although age ranged from 8 to 60 years, the effect of age was

assessed (van Rijn et al., 2018). Social cognition was assessed using computerized tasks of pattern identification, face recognition, and facial emotion recognition. Accuracy in performance in the XXY group differed from the control group specifically when stimuli were of a more social nature (i.e., during facial emotion recognition). The XXY group on average needed more time to identify facial expressions, although performance accuracy did not increase with more time. The results were independent of age, suggesting that the difficulties with emotion recognition are already apparent during childhood. The second study used the same computerized tasks to study face processing and emotion recognition skills in in two groups of boys with XXY from the Netherlands ($N = 44$) and from the United States ($N = 54$) (Samango-Sprouse et al., 2018). Developmental risk was calculated as a percentage of children that scored in the significantly impaired range (i.e., $Z > 2.0$). Results showed that 23-25% of the children experienced significant and clinically relevant difficulties with face processing. In addition, 16-44% of the children experienced significant and clinically relevant difficulties with emotion recognition (i.e., identifying sad, happy, or angry emotions) The third study tested a group of 46 boys and girls with an extra X chromosome, between the ages of 9-18 years (van Rijn, Stockmann, van Buggenhout, et al., 2014). Measures included assessments of ToM and emotion recognition. Children with an extra X chromosome performed more poorly on the ToM task than the control group. In addition, on average children with an extra X chromosome showed difficulties in the ability to identify emotional faces which was expressed in the reduced accuracy, rather than reaction times, and most prominent for angry faces. No differences were found in the performance of the XXX versus the XXY group, nor in the performance of children in the prenatal follow-up versus the referred group.

Table 2. Included studies global intellectual functioning

Authors	*N*	Age	Comparison	Subdomain(s)	Instrument(s)	Results
Ross et al., 2008	47 XXY	4-9;11 years 10-17;8 years	Normed scores	GCA	DAS	Older boys < younger boys
Ross et al., 2009	93 XXY 21 XYY	4-18 years	Control group	GCA VP NVP Spatial cluster	DAS	XXY = XYY < controls XXY = XYY < controls XXY = XYY < controls XXY < XYY = controls
Cordeiro et al., 2012	95 XXY 29 XYY	4-18 years	Normed scores	VIQ-PIQ Gap	DAS, WASI or WISC	XXY VIQ < PIQ XYY VIQ < PIQ
Bruining et al., 2009	47 XXY	6-19 years	Normed scores	FSIQ PIQ VIQ	WISC or WASI	XXY < controls XXY < controls XXY < controls
Ratcliffe, 1999	19 XXY 19 XYY 16 XXX	6-8 years	Control group	PIQ VIQ	WISC	XXY < controls XYY < controls XXX < controls XXY < controls XYY < controls XXX < controls
Rovet et al., 1995; 1996	21-29 XXY	6-18 years	Control group	PIQ VIQ VIQ-PIQ Gap	WISC or WASI	XXY < controls XXY < controls XXY VIQ < PIQ
Netley, 1986	73 XXY 32 XXX 28 XYY	*M*xxy = 10.3 years *M*xxx = 10.5 years *M*xyy = 9.5 years	Normed scores	FSIQ PIQ VIQ VIQ-PIQ Gap	WISC or WASI	XXY < controls XXX < controls XYY n.s. XXY n.s. XYY n.s. XXX < controls XXY < controls XXX < controls XYY n.s. XXX VIQ < PIQ

Abbreviations: DAS, Differential Ability Scales; FSIQ, full scale intelligence quotient; GCA, General Conceptual Ability; n.s., no significant differences; IQ, intelligence; NVP, Nonverbal Performance; PIQ, performance intelligence quotient; WASI, Wechsler Abbreviated Scale of Intelligence; VIQ, verbal intelligence quotient; VP, Verbal Performance; WISC, Wechsler Intelligence Scale for Children.

Table 3. Included studies language domain and calculated effect sizes

Authors	*N*	Age	Comparison	Subdomain(s)	Instrument(s) + Type(s)	Results	Effect Sizes
Zampini et al., 2018	15 XXX /XXY	24 months	Control group	Vocabulary size	CDI (P)	XXX/XXY < controls	d = 2.18***
				Verbal productions	Structured-play session (O)	XXX/XXY < controls	d_{range} = .99-1.44***
				Number of Utterances		XXX/XXY < controls	d_{range} = 1.76-2.08***
				Pointing gestures		XXX/XXY > controls	d = 1.03***
Haka-Ikse et al., 1978	25 XXY	36-72 months	Normed scores	Language difficulties	YDS (P)	>50%	N/A
Ross et al., 2008	47 XXY	4-9;11 years 10-17;8 years	Normed scores	Complex levels of language processing	TLC-E (C)	XXY < controls; Older boys < younger boys	d = 1.45***
				Expressive vocabulary	EOWPVT (C)	n.s.	
				Receptive vocabulary	ROWPVT (C)	n.s.	
				Semantic fluency	DKEFs (C)	n.s.	
				Phonetic fluency		n.s.	
				Phonological processing	CTOPP (C)	n.s.	
Ross et al., 2009	93 XXY 21 XYY	4-18 years	Control group	Receptive vocabulary	ROWPVT (C)	XYY < XXY < controls	d_{xxy} = 1.15*** d_{xyy} = 1.85***
				Complex levels of language processing	TLC-E (C)	XXY = XYY < controls	d_{xxy} = 1.63*** d_{xyy} = 1.33***
				Expressive vocabulary	EOWPVT (C)	XXY = XYY < controls	d_{xxy} = .96*** d_{xyy} = 1.17***
				Phonetic fluency	DKEFs (C)	XXY = XYY < controls	d_{xxy} = .97*** d_{xyy} = 1.08***
				Phonological processing	CTOPP (C)	Inconclusive results	
				Semantic fluency	DKEFs (C)	n.s.	
Bishop et al., 2011	58 XXX 19 XXY 58 XYY	4-17 years	Control group	Structural and pragmatic difficulties	CCC (P)	XXX 44-68% XXY 50% XYY 38-85%	N/A

Note: *** High clinical significance; ** Moderate clinical significance; * Low clinical significance; N/A, not applicable; n.s., no significant differences.
Abbreviations: C, Performance Task Child; CCC, Children's Communication Checklist; CDI, MacArthur Communicative Development Inventories; CTOPP, Comprehensive Test of Phonological Processing; DKEFs, Delis-Kaplin Executive Function system; EOWPVT, Expressive One-Word Picture Vocabulary Test; O, Observation; P, Parent Report; ROWPVT, Receptive One Word Picture Vocabulary Test; TLC-EL, Test of Language Competence—Expanded Edition; YDS, Yale Developmental Schedules.

Table 4. Included studies executive functioning domain and calculated effect sizes

Authors	*N*	Age	Comparison	Subdomain(s)	Instrument(s) + Type(s)	Results	Effect Sizes
Lee et al., 2015	15 XXY 15 XXX	5-18 years	Control group	Daily life executive functioning	BRIEF (P)	XXX/XXY > controls[a]	N/A
Ross et al., 2008	47 XXY	4-18 years	Normed scores	Sustained attention – omissions	C(K)CPT (C)	XXY > controls[a]	N/A
				Sustained attention – variability		XXY > controls[a]	N/A
				Sustained attention – reaction time		XXY > controls[a]	N/A
				Inhibition	DKEFS-CWIT (C)	n.s.	
				Mental flexibility		n.s.	
Ross et al., 2009	93 XXY 21 XYY	4-18 years	Control group	Sustained attention - omissions	C(K)CPT (C)	XXY > XYY = controls[a]	d_{xxy} = .83***
				Sustained attention – variability		XXY = XYY > controls[a]	d_{xxy} = .80*** d_{xyy} = .86***
				Sustained attention – reaction time		XXY = XYY > controls[a]	d_{xxy} = 1.02*** d_{xyy} = 1.04***
				Sustained attention – commissions		n.s.	
				Inhibition	DKEFS-CWIT (C)	XYY < XXY < controls	d_{xyy} = 1.09***
				Mental flexibility		XYY < XXY < controls	d_{xyy} = 1.71***
Van Rijn & Swaab, 2015	40 XXX/XXY	9-18 years	Control group	Sustained attentional control	ANT (C)	XXX/XXY < controls	*d* = .33*
				Inhibition		XXX/XXY < controls	*d* = .38*
				Mental flexibility		XXX/XXY < controls	*d* = .45*
				Visual working memory		XXX/XXY < controls	*d* = .68**
				Focused attention		n.s.	
				Verbal working memory		n.s.	
				Daily life executive functioning	DEX (P)	XXX/XXY < controls	*d* = 1.37***
Samango-Sprouse et al., 2018	44 XXY (NL) 54 XXY (USA)	8-18 years	Normed scores	Sustained attention; % significant impaired	ANT (C)	19-57%	N/A
				Inhibition; % significant impaired		26-28%	N/A
				Mental flexibility; % significant impaired		35-36%	N/A

Note: *** High clinical significance; ** Moderate clinical significance; * Low clinical significance; N/A, not applicable; n.s., not significant; [a] higher scores denote more problems.
Abbreviations: ANT, Amsterdam Neuropsychological Tasks; BRIEF, Behavior Rating Inventory of Executive Function; C, Performance Task Child; C(K)CPT, Conners' (Kiddie) Continuous Performance Test; DEX, Dysexecutive Questionnaire; DKEFS-CWIT; Delis-Kaplin Executive Functioning Color-Word Interference Test; P, Parent Report.

Table 5. Included studies social cognition domain and calculated effect sizes

Authors	*N*	Age	Comparison	Subdomain(s)	Instrument(s) + Type(s)	Results	Effect Sizes
Ross et al., 2015	18 XYY	4-14 years	Control group	Social cognition	SRS (P)	XYY > controls[a]	d = .68**
Cordeiro et al., 2012	102 XXY 40 XYY	4-18 years	Normed scores	Social cognition	SRS (P)	XYY > XXY > controls[a]	d_{xxy} = .93*** d_{xyy} = 1.80***
Van Rijn et al., 2014a	60 XXX/XXY	9-18 years	Control group	Social cognition	SRS (P)	XXX/XXY > controls[a]	d = 1.61***
Van Rijn et al., 2018	70 XXY	8-60 years	Normed scores	Pattern recognition – Reaction time % impaired	ANT (C)	17%	N/A
				Pattern recognition – Accuracy % impaired		9%	N/A
				Face processing – Reaction time % impaired		26%	N/A
				Face processing – Accuracy % impaired		13%	N/A
				Facial emotion recognition – Reaction time % impaired		33%	η^2 = .40***
				Facial emotion recognition – Accuracy % impaired		13%	η^2 = .16**
Samango-Sprouse et al., 2018	44 XXY (NL) 54 XXY (USA)	8-18 years	Normed scores	Face processing - % impaired	ANT (C)	23-25%	N/A
				Facial emotion recognition - % impaired		16-44%	N/A
Van Rijn et al., 2014b	46 XXX/XXY	9-18 years	Control group	Theory of Mind – egocentric role taking	SCST (C)	XXX/XXY < controls	d = .85***
				Theory of Mind - subjective role taking		XXX/XXY < controls	d = 1.03***
				Theory of Mind - self-reflective role taking		XXX/XXY < controls	d = .69**
				Theory of Mind - mutual role taking		XXX/XXY < controls	d = .83***
				Facial affect identification – angry faces	KDEF (P)	XXX/XXY < controls	d = 3.30***

Note: *** High clinical significance; ** Moderate clinical significance; * Low clinical significance; N/A, not applicable; n.s., not significant; [a] higher scores denote more problems.
Abbreviations: ANT, Amsterdam Neuropsychological Tests; C, Performance Task Child; KDEF, Karolinska Directed Emotional Faces; P, Parent Report; SCST, Social Cognitive Skills Tests; SRS, Social Responsiveness Scale; ToM,rt, Theory of Mind role taking.

Discussion

The aim of this review was two-fold. The first aim was to review to what degree impairments in areas of global intellectual functioning, language development, social cognition, and executive functioning have been studied in children with SCT and identify possible gaps in research that future research should focus on. The second aim was to establish clinical significance of these impairments and identify risk-factors that should be closely monitored from early development onwards or that should be included in standard clinical neuropsychological screening to identify potential targets for support and intervention.

With regard to the first aim, the reviewed studies collectively gave the following results. On the domain of global intellectual functioning (GIF), eight studies report outcomes in children between the ages of 4-18 years, with three studies focusing on children from the age of four years, and four studies studying school-aged children. To our knowledge, there were no studies that examined GIF in children with SCT before the age of four years. On the domain of language development, five studies reported outcomes in children between the ages of 2-18 years. To our knowledge, there were no studies that examined language development in children with SCT before the age of two years. Of the five studies, two studies used only parent reports, the other three studies used either a performance task or a combination of parent report and performance tasks. On the domain of executive functioning, five studies reported outcomes in children between the ages of 4-18 years. To our knowledge, there are no studies to date that assess (precursors of) executive functioning in children with SCT before the age of four years. In addition, all studies included children with XXY; two studies also included girls with XXX, and one study also included boys with XYY. Finally, one study used parent report, with the other four studies using performance-based tasks or a combination of both. On the domain of social cognition, six studies reported outcomes in children between the ages of 4-18 years. To our knowledge, there are no studies to date that assess (precursors of) social cognition in children with SCT before the age of four years. In addition, until the age of eight years, and in XXX and XXY groups only, social cognition has not been tested with performance-based measures but has solely been assessed with parent reports. To this date, no studies have reported child-data on social cognition in boys with XYY. Taken together, although GIF and language have received relatively much attention, there is a great need for more studies in areas of executive functioning and social cognition in children with SCT. Also, research should rely more on performance-based measures in addition to parent report. Finally, we stress the importance of following children over time. Longitudinal studies are needed to keep an eye on the developmental trajectory and could help determine which difficulties in early life are predictive of outcomes in later life.

With regard to the second aim, the researched studies collectively gave the following result. On the domain of global intellectual functioning, from the age of four years there appears to be a general finding that the GIF of children with SCT is variable, and ranges from impaired to above average with mean GIF in the average to low-average range. There might be to be some differences between the three karyotypes, with XXX girls showing reductions in both VIQ and PIQ, XXY boys showing reduced VIQ compared to PIQ, and XYY boys functioning variably. On the domain of language development, it appears that language difficulties can

already be detected during the toddler-age and can be persistent throughout adolescence. Difficulties with language development have not only been reported by parents but have also been observed during language assessments. All calculated effect sizes indicated high clinical significance, stressing the need for early detection and support programs on the domain of language. Especially complex levels of language, such as semantics, syntax, and pragmatics seem to be impaired. In addition, one study reported that older children appear to experience more difficulties than younger children. It is possible that children experience more (severe) difficulties, or that problems become more apparent during a certain age because of different task demands. A possible explanation for this is the phenomenon of 'growing into deficit'; which occurs when age increases, while the expected rate of progress stays behind, resulting in a growing deficit (as compared with typically developing peers), and a growing impact on daily life (Rourke et al., 1983). The reported language difficulties appear to be somewhat similar in girls with XXX and boys with XXY. Only one study compared boys with XXY and XYY, with XYY boys experiencing more difficulties in receptive vocabulary, but performing similarly with XXY boys on other areas of language development. On the domain of executive functioning, two studies indicated that parents of children with SCT report more difficulties with executive functioning. For one of these studies, we were able to calculate an effect size, which indicated high clinical significance. The studies that used performance-based tasks report somewhat variable outcomes, partially depending on the included participant groups. All five studies included boys with XXY and have reported poorer performance and/or more difficulties when compared to controls, effect sizes were calculated for two of these studies, with one study indicating high clinical significance on the subdomain of sustained attention, inhibition, and mental flexibility, whereas the other study, which included slightly older children, indicated low to moderate clinical significance on these domains. Two studies included girls with XXX (in combination with boys with XXY) and reported poorer performance and/or more difficulties when compared to controls on the subdomains of sustained attentional control, inhibition, mental flexibility, and visual working memory, effect sizes indicated low to moderate clinical significance. One study included boys with XYY and reported more variability and longer reaction times on tasks that measure sustained attention. Effect sizes indicated high clinical significance. On the domain of social cognition, three studies indicated that parents of children with SCT report more difficulties with social cognition. Calculated effect sizes for all three studies indicated high clinical significance. One studies that used a performance-based task reported difficulties in boys with XXY on the subdomain of Theory of Mind; with effect size indicating high clinical significance. Three of the studies that included boys with XXY reported difficulties with facial emotion recognition, with effect sizes indicating high clinical significance. One study included girls with XXX (in combination with boys with XXY) and reported poorer performance on facial effect identification, in particular when identifying angry faces. Calculated effect sized indicate very high clinical significance.

In conclusion, from a developmental perspective it is important to monitor neuropsychological functioning of children with SCT at the start, or even before, the sensitive developmental period when these skills typically develop, and identify precursors and early markers of developmental risk. Considering the increased prevalence of (characteristics of) behavioral and neurodevelopmental disorders, such as ADHD, autism spectrum disorders,

anxiety, and depression in the SCT population (Ross et al., 2012; Tartaglia et al., 2012; Van Rijn, 2018), more knowledge of developmental neurocognitive risk markers could lead to more timely, preventive support, hopefully reducing the risk for these behavioral and neurodevelopmental disorders in the future. In addition, the results of this review call for more studies on early neurocognitive vulnerabilities, which are expected based on the impact of the extra chromosome on the development of the brain (Printzlau et al., 2017). It is important to learn more about the involvement of genes on the sex chromosomes in order to identify how expression of these genes can lead to the behavioral phenotype of individuals with SCT and how different genes on different sex chromosomes can lead to the similarities and differences in the behavioral profile of children with XXX, XXY, and XYY. There is a specific need for more knowledge in areas in executive functioning and social cognition, not only because more extensive research has shown these domains appear to be affected in adulthood (Van Rijn, 2018), but also because these cognitive domains are crucial for behavioral and socio-emotional development, adaptive functioning, and quality of life. Also, the results of this review illustrate that more attention should be given to timely screening for cognitive vulnerabilities, that these should be monitored during relevant developmental stages, and that interventions should be tailored to these risk profiles.

Finally, it is also important to gain more insight in the karyotype-specific profiles of neurocognitive functioning, as the presence of an extra X or Y may have similar *and* different effects on development of brain areas involved in social cognition and language, and therefore could have effect on neurocognitive development. This may help in understanding expected neurodevelopmental profiles and related, tailored, intervention options.

Recruitment strategy will always lead to variance in the SCT phenotype with overestimation of some difficulties (e.g., because these difficulties led to genetic screening in postnatally diagnosed individuals), whereas other difficulties may be underestimated (e.g., because prenatally diagnosed individuals may have benefited from early preventive support, such as speech therapy). For that reason, it is difficult to assess the full spectrum of strengths and weaknesses in individuals with SCT when using only one strategy. By including all studies regardless of the used recruitment strategy, we have attempted to balance bias, even though the described outcomes may not be fully representative for the total population children with SCT.

To conclude, this review of studies shows that the presence of an extra sex chromosome may have impact on neurocognitive functioning of children with SCT, and that domains of language development, executive functioning, and social cognition should be closely monitored in these children. In addition, it is important to gain more insight in the early development of children with SCT population, especially before the age of four years on the domains of social cognition and executive functioning. Finally, it is important that social cognition and executive functioning will be included in the standard screening and assessment methods, as this review showed that social cognition and executive functioning in addition to language development, are domains that require close monitoring, and are targets for early support and intervention programs. With more knowledge about the development of young children with SCT, existing evidence-based (preventive) intervention programs can be tailored to the SCT profile in hopes

of reducing these difficulties, and by reducing these neurocognitive underpinnings of behavior, could possibly prevent neurobehavioral problems in later life.

References

Abramsky, L., & Chapple, J. (1997). 47,XXY (Klinefelter syndrome) and 47,XYY: Estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenatal Diagnosis*, *17*(4), 363-368. https://doi.org/10.1002/(sici)1097-0223(199704)17:4%3C363::aid-pd79%3E3.0.co;2-o

Andersen, S. L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? [Article; Proceedings Paper]. *Neuroscience and Biobehavioral Reviews*, *27*(1-2), 3-18. https://doi.org/10.1016/s0149-7634(03)00005-8

Bishop, D. V. M., Jacobs, P. A., Lachlan, K., Wellesley, D., Barnicoat, A., Boyd, P. A., . . . Scerif, G. (2011). Autism, language and communication in children with sex chromosome trisomies. *Archives of Disease in Childhood*, *96*(10), 954-959. https://doi.org/10.1136/adc.2009.179747

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevelance of Klinefelter syndrome: A national registry study. *The Journal of Clinical Endocrinology & Metabolism*, *88*(2), 622-626. https://doi.org/10.1210/jc.2002-021491

Bruining, H., Swaab, H., Kas, M., & van Engeland, H. (2009). Psychiatric characteristics in a self-selected sample of boys with Klinefelter syndrome. *Pediatrics*, *123*(5), e865-870. https://doi.org/10.1542/peds.2008-1954

Cordeiro, L., Tartaglia, N., Roeltgen, D., & Ross, J. (2012). Social deficits in male children and adolescents with sex chromosome aneuploidy: A comparison of XXY, XYY, and XXYY syndromes. *Research in Developmental Disabilities*, *33*(4), 1254-1263. https://doi.org/10.1016/j.ridd.2012.02.013

Giltay, J., & Maiburg, M. (2010). Klinefelter syndrome: Clinical and molecular aspects. *Expert Review of Molecular Diagnostics*, *10*, 765-776. https://doi.org/10.1586/erm.10.63

Groth, K. A., Skakkebaek, A., Høst, C., Gravholt, C. H., & Bojesen, A. (2013). Klinefelter syndrome - a clinical update. *The Journal of Clinical Endocrinology & Metabolism*, *98*(1), 20-30. https://doi.org/10.1210/jc.2012-2382

Haka-Ikse, K., Stewart, D., & Cripps, M. H. (1978). Early development of children with sex chromosome aberrations. *Pediatrics*, *62*(5), 761-766.

Hong, D. S., & Reiss, A. L. (2014). Cognitive and neurological aspects of sex chromosome aneuploidies. *The Lancet Neurology*, *13*(3), 306-318. https://doi.org/10.1016/s1474-4422(13)70302-8

Lee, N. R., Anand, P., Will, E., Adeyemi, E. I., Clasen, L. S., Blumenthal, J. D., . . . Edgin, J. O. (2015). Everyday executive functions in Down syndrome from early childhood to young adulthood: evidence for both unique and shared characteristics compared to youth with sex chromosome trisomy (XXX and XXY). *Frontiers in Behavioral Neuroscience*, *9*, 264, Article 264. https://doi.org/10.3389/fnbeh.2015.00264

Leggett, V., Jacobs, P., Nation, K., Scerif, G., & Bishop, D. V. (2010). Neurocognitive outcomes of individuals with a sex chromosome trisomy: XXX, XYY, or XXY: A systematic review. *Developmental Medicine & Child Neurology*, *52*(2), 119-129. https://doi.org/10.1111/j.1469-8749.2009.03545.x

Lenroot, R. K., Blumenthal, J. D., Wallace, G. L., Clasen, L. S., Lee, N. R., & Giedd, J. N. (2014). A case-control study of brain structure and behavioral characteristics in 47,XXX syndrome. *Genes Brain and Behavior*, *13*(8), 841-849. https://doi.org/10.1111/gbb.12180

Morris, J. K., Alberman, E., Scott, C., & Jacobs, P. (2008). Is the prevalence of Klinefelter syndrome increasing? *European Journal of Human Genetics*, *16*, 163-170. https://doi.org/10.1038/sj.ejhg.5201956

Netley, C. T. (1986). Summary overview of behavioural development in individuals with neonatally identified X and Y aneuploidy. In S. G. Ratcliffe & N. Paul (Eds.), *Prospective studies on children with sex chromosome aneuploidy* (Vol. 22, pp. 293-306). Alan R. Liss, Inc.

Otter, M., Schrander-Stumpel, C. T., & Curfs, L. M. (2010). Triple X syndrome: A review of the literature. *European Journal of Human Genetics*, *18*(3), 265-271. https://doi.org/10.1038/ejhg.2009.109

Printzlau, F., Wolstencroft, J., & Skuse, D. H. (2017). Cognitive, behavioral, and neural consequences of sex chromosome aneuploidy. *Journal of Neuroscience Research*, *95*(1-2), 311-319. https://doi.org/10.1002/jnr.23951

Ratcliffe, S. (1999). Long term outcome in children of sex chromosome abnormalities. *Archives of Disease in Childhood*, *80*, 192-195.

Ross, J. L., Roeltgen, D. P., Kushner, H., Zinn, A. R., Reiss, A., Bardsley, M. Z., . . . Tartaglia, N. (2012). Behavioral and social phenotypes in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Pediatrics*, *129*(4), 769-778. https://doi.org/10.1542/peds.2011-0719

Ross, J. L., Roeltgen, D. P., Stefanatos, G., Benecke, R., Zeger, M. P., Kushner, H., . . . Zinn, A. R. (2008). Cognitive and motor development during childhood in boys with Klinefelter syndrome. *American Journal of Medical Genetics Part A*, *146A*(6), 708-719. https://doi.org/10.1002/ajmg.a.32232

Ross, J. L., Tartaglia, N., Merry, D. E., Dalva, M., & Zinn, A. R. (2015). Behavioral phenotypes in males with XYY and possible role of increased NLGN4Y expression in autism features. *Genes Brain Behav*, *14*(2), 137-144. https://doi.org/10.1111/gbb.12200

Ross, J. L., Zeger, M. P., Kushner, H., Zinn, A. R., & Roeltgen, D. P. (2009). An extra X or Y chromosome: contrasting the cognitive and motor phenotypes in childhood in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Developmental Disabilities Research Reviews*, *15*(4), 309-317. https://doi.org/10.1002/ddrr.85

Rourke, B. P., Bakker, D. J., Fisk, J. L., & Strang, J. D. (1983). *Child Neuropsychology*. Guilford Press.

Rovet, J., Netley, C., Bailey, J., Keenan, M., & Stewart, D. (1995). Intelligence and achievement in children with extra X aneuploidy: A longitudinal perspective. *American Journal of Medical Genetics*, *60*, 356-363.

Rovet, J., Netley, C., Keenan, M., Bailey, J., & Stewart, D. (1996). The psychoeducational profile of boys with Klinefelter syndrome. *Journal of Learning Disabilities*, *29*(2), 180-196.

Samango-Sprouse, C. A., Keen, C., Sadeghin, T., & Gropman, A. L. (2017). The benefits and limitations of cell-free DNA screening for 47,XXY (Klinefelter syndrome). *Prenatal Diagnosis*, *37*(5), 497-501. https://doi.org/10.1002/pd.5044

Samango-Sprouse, C. A., Stapleton, E., Chea, S., Lawson, P., Sadeghin, T., Cappello, C., . . . Van Rijn, S. (2018). International investigation of neurocognitive and behavioral phenotype in 47,XXY (Klinefelter syndrome): Predicting individual differences. *American Journal of Medical Genetics*, *176*, 877-885. https://doi.org/10.1002/ajmg.a.38621

Tartaglia, N. R., Ayari, N., Hutaff-Lee, C., & Boada, R. (2012). Attention-deficit hyperactivity disorder symptoms in children and adolescents with sex chromosome aneuploidy: XXY, XXX, XYY, and XXYY. *Journal of Developmental and Behavioral Pediatrics*, *33*(4), 309-318. https://doi.org/10.1097/DBP.0b013e31824501c8

Tartaglia, N. R., Howell, S., Sutherland, A., Wilson, R., & Wilson, L. (2010). A review of trisomy X (47,XXX). *Orphanet Journal of Rare Diseases*, *5*, 8. https://doi.org/10.1186/1750-1172-5-8

Van Rijn, S. (2018). A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47,XXX, 47,XYY). *Current Opinion in Psychiatry*, *32*. https://doi.org/10.1097/yco.0000000000000471

Van Rijn, S., de Sonneville, L., & Swaab, H. (2018). The nature of social cognitive deficits in children and adults with Klinefelter syndrome. *Genes Brain and Behavior*, *17*(6), e12465. https://doi.org/10.1111/gbb.12465

Van Rijn, S., Stockmann, L., Borghgraef, M., Bruining, H., van Ravenswaaij-Arts, C., Govaerts, L., . . . Swaab, H. (2014). The social behavioral phenotype in boys and girls with an extra X chromosome (Klinefelter syndrome and Trisomy X): A comparison with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *44*(2), 310-320. https://doi.org/10.1007/s10803-013-1860-5

Van Rijn, S., Stockmann, L., van Buggenhout, G., van Ravenswaaij-Arts, C., & Swaab, H. (2014). Social cognition and underlying cognitive mechanisms in children with an extra X chromosome: a comparison with autism spectrum disorder. *Genes Brain Behav*, *13*(5), 459-467. https://doi.org/10.1111/gbb.12134

Van Rijn, S., & Swaab, H. (2015). Executive dysfunction and the relation with behavioral problems in children with 47,XXY and 47,XXX. *Genes Brain and Behavior*, *14*(2), 200-208. https://doi.org/10.1111/gbb.12203

Wigby, K., D'Epagnier, C., Howell, S., Reicks, A., Wilson, R., Cordeiro, L., & Tartaglia, N. (2016). Expanding the phenotype of Triple X syndrome: A comparison of prenatal versus postnatal diagnosis. *American Journal of Medical Genetics Part A*, *170*(11), 2870-2881. https://doi.org/10.1002/ajmg.a.37688

Zampini, L., Draghi, L., Silibello, G., Dall'Ara, F., Rigamonti, C., Suttora, C., . . . Vizziello, P. (2018). Vocal and gestural productions of 24-month-old children with sex chromosome trisomies. *International Journal of Language & Communication Disorders*, *53*(1), 171-181. https://doi.org/10.1111/1460-6984.12334

Chapter 4

A cross-sectional study of early language abilities in children with sex chromosome trisomy (XXY, XXX, XYY) aged 1-6 years

Published as:

Urbanus, E., Swaab, H., Tartaglia, N., Boada, R., & Van Rijn, S. (2022). A cross-sectional study of early language abilities in children with sex chromosome trisomy (XXY, XXX, XYY) aged 1-6 years. *Child Neuropsychology, 28*(2), 171-196. https://doi.org/10.1080/09297049.2021.1960959

Abstract

Children with sex chromosome trisomy (SCT) are at increased risk for developing language difficulties. Earlier studies have reported that as many as 70-80% of individuals with SCT show some form of language difficulties. Language develops rapidly in the first years of life; knowledge about language development at an early age is needed. The present study aims to identify the language abilities of young children with SCT across multiple language domains and to identify the percentage of children that, according to clinical guidelines, have language difficulties.

Children between the ages of 1-6-years (N_{SCT}=103, $N_{controls}$=102) were included. Nonverbal communication, early vocabulary, semantic, syntax, and phonological skills were assessed.

Language difficulties were already present in 1-year-old children with SCT and across the age range in various language domains. Clinical classification showed that, depending on the assessed domain, 14.8-50.0% of the children scored below the 16th percentile. There was no effect of time of diagnosis, ascertainment bias, research site, nor SCT specific karyotype (i.e., XXX vs XXY, vs XYY) on language outcomes.

Overall, language difficulties can already be present in very young children with SCT. These findings appear to be robust within the SCT group and are found in various language domains. These results highlight the importance of monitoring both receptive and expressive language development already at the earliest stages of nonverbal communication. Finally, as early language skills are the building blocks for later social communication, literacy, and self-expression, longitudinal studies that investigate the effect of early interventions on later language outcomes are warranted.

Introduction

Sex chromosome trisomy (SCT), the presence of an extra X or Y chromosome leading to a XXX, XXY, or XYY karyotype, is caused by a spontaneous error during early cell division (Leggett et al., 2010). With an estimated prevalence of 1:650 to 1:1000 live births (Bojesen et al., 2003; Groth et al., 2013; Morris et al., 2008), SCT is one of the most common chromosomal duplications in humans. The presence of an extra X or Y chromosome can impact neurocognitive development in children (for a review see Urbanus et al., 2019), and previous studies have shown that individuals with SCT have an increased risk for neurodevelopmental disorders (for a review see Van Rijn, 2019), and behavioral problems (Urbanus et al., 2020).

One of the most distinctive traits of SCT is the impact the extra chromosome may have on language development. Previous studies have reported that as many as 70-80% of included individuals with SCT has some form of language difficulty (for a review see e.g., Boada et al., 2009; Leggett et al., 2010; Robinson et al., 1983). Most of these studies have included school-aged children or adolescents, with only a handful of studies including small samples of children under the age of four years, a time when language develops rapidly. Language development plays an important role in cognitive and social development (Simms, 2007), and is required for communication of one's needs, thoughts, and emotions. In addition, language is needed for learning and evaluation, for example in helping us to reflect upon what we experience and helping us understand the world around us. Language is also critical for reading and literacy. If language develops poorly, this can have severe consequences for other developmental domains (e.g., cognitive and emotional development), consequently also affecting one's ability to participate in society, or the experienced quality of life.

Typically, before young children are able to use spoken language, children use gestures to communicate with others (i.e., *early nonverbal communication*). With increasing age, children start to understand the meaning of perceived words, sentences, and conversations (i.e., the development of *receptive* language), and then they start to use spoken language (i.e., *expressive language*) to convey meaning and thoughts through the production of words and sentences, as they engage in conversation (Levey, 2019). Children need to develop certain language skills to acquire adequate linguistic competence. The distinction between the following skills can be made: 1) Phonology (how sounds form a word), 2) morphology (how words are formed), 3) syntax (how words are combined to form sentences), 4) semantics (specific meaning of words, phrases, and sentences; including *lexicon* or vocabulary), and 5) pragmatics (use of language in a social setting; Owens Jr., 2011) .

Although not much is known about the first few years of language development in SCT, review studies, which cover results from both prospective newborn screening studies and more recent research and include individuals regardless of time of diagnosis (i.e., prenatal or postnatal), generally report difficulties in one or more of the language domains. Overall, within the SCT group as a whole, studies report difficulties with language already at a young age. Language difficulties are both reported by parents as well as demonstrated in task performances of included children. Generally, studies reported large effect sizes, ranging from .96 to 2.18 (Cohen's *d*), indicating high clinical significance of language difficulties (Urbanus et al., 2019). For school-aged girls with XXX, the results overall show an increased risk for early

developmental speech and language difficulties (Leggett et al., 2010), with expressive language possibly more affected than receptive language (Tartaglia et al., 2010). Fifty to 75% of girls show compromised receptive and expressive language (Otter et al., 2010). Language problems often continue in adolescence and young adulthood, and therefore continue to interfere with overall functioning (Otter et al., 2010; Tartaglia et al., 2010). For school-aged boys with XXY, the results overall show compromised speech and language development (Boada et al., 2009), with language difficulties occurring in 70-80% of the children (Boada et al., 2009; Geschwind et al., 2000). Similar to girls with XXX, expressive language appears to be more severely affected than receptive language in boys with XXY (Leggett et al., 2010; Visootsak & Graham Jr., 2006). There is evidence for general language impairments of a persistent nature (Hong & Reiss, 2014; Verri et al., 2010), with difficulties becoming more prominent with increasing age (Geschwind et al., 2000; Mandoki et al., 1991). For boys with XYY, information is limited. Re and Birkhoff (2015) report compromised speech and language development in childhood, and Leggett et al. (2010) report mixed findings, indicating that more research is needed.

Collectively the studies included in the reviews demonstrate that atypical language development is common in individuals with SCT, and that persistent language impairment may influence quality of life. However, most of these findings are based on studies including school-aged children, adolescents, or adults, and both the number of the included individuals and the recruitment strategy (e.g., prospective follow-up, clinical-, or research groups) of the group varied from study from study, making it difficult to generalize results. Only a few previous studies have focused on very young children with SCT (Zampini et al., 2020; Zampini et al., 2017; Zampini et al., 2018). To understand the emergence and trajectory of developmental language problems, it is important to assess language abilities in infancy and toddlerhood at the early stages of rapid development and to assess multiple language domains at different developmental stages. This stresses the need for studies focusing on the first years of life, in order to identify children at risk for language difficulties and to detect early markers of aberrant language development. The present study focuses on the first six years of life; a time where several important milestones within child development occur, starting from a period where children mostly rely on nonverbal communication and start to use words to a period where children start learning in school.

It is important to explore if signs of difficulties in language development can already be identified in very young children with SCT. As there is significant brain growth in the first three years of life and language difficulties have shown to be persistent across the life span, early detection of risk in language development could support the need for the development of tailored support programs and early preventive intervention to mitigate worse outcomes later in life.

This study evaluates a range of language outcomes in children with SCT, more specifically this study focuses on the use of early non-verbal communication (i.e., gestures), early vocabulary, semantics, syntax, and phonological processing skills. Factors that could contribute to individual differences in language abilities in the SCT population, were assessed, this included specific SCT karyotype (i.e., XXX vs XXY, vs XXY), timing of diagnosis, ascertainment bias, and research site. Recognizing that language develops dynamically during

early childhood, the core goal of this study is to investigate the role of age in the language abilities of children with SCT. Specifically, this study aims to identify the language abilities of children with SCT at different developmental stages; to describe the variability within these abilities; and to identify the proportion of children who, according to clinical guidelines, have language difficulties.

Materials and Methods

Participants

The present study is part of a larger ongoing project (TRIXY Early Childhood Study). The TRIXY Early Childhood Study is a longitudinal study that included children with and without SCT aged 1-7 years and aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. For the present study, children aged 1-6 years were included; only results from the first visit are reported.

In total, 205 children participated in the present study, 103 children with SCT and 102 children without SCT. Ages ranged from 11 months to 6 years and 11 months (see Table 1 for descriptives of the groups). Of the 103 children with SCT, 70 children received a prenatal diagnosis with genetic testing performed due to routine prenatal screening or advanced maternal age. Of the 33 children who received a postnatal diagnosis, 14 received the diagnosis because of a developmental delay (including language delays), ten because of physical and/or growth problems (e.g., small testes), and nine because of medical concerns or suspicion of other genetic syndromes. Within the XXY-group, 24 children (49%) had received early testosterone supplements.

Table 1. Descriptives SCT versus controls

	SCT	XXX	XXY	XYY	Control	XX	XY	*p* (SCT vs Control)	SCT comparisons
Total *N*	103	32	49	22	102	58	44		
Age	3.54 (1.83)	4.17 (1.69)	3.16 (1.85)	3.47 (1.80)	3.60 (1.62)	3.63 (1.62)	3.56 (1.63)	.785	XXX = XXY = XYY
GIF[a]	97.45 (17.01)	94.90 (16.56)	100.42 (16.65)	94.26 (18.24)	105.70 (14.34)	104.19 (13.57)	107.68 (15.23)	<.001	XXX = XXY = XYY
SES[b]	5.93 (.94)	5.94 (1.03)	6.05 (.88)	5.66 (.92)	5.43 (1.40)	5.24 (1.33)	5.68 (1.47)	.003	XXX = XXY = XYY

Note: scores represent Means (SD)
SCT = Sex Chromosome Trisomy; SCT comparisons = XXX versus XXY versus XYY; GIF = level of global intellectual functioning; SES = socioeconomic status
[a] Data for 6 children with SCT was incomplete
[b] Data for 1 child with SCT was missing

Recruitment and assessment took place at the Trisomy of the X and Y chromosomes (TRIXY) Expert Center in the Netherlands and at the eXtraordinarY Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado in the USA. With the help of clinical genetics departments (from the Netherlands, the Dutch speaking parts in Belgium, and Colorado), pediatricians, and national advocacy or support groups for individuals with SCT children in the SCT group were recruited by sending out recruitment flyers and with postings on the internet (e.g., TRIXY website and the eXtraordinarY Kids Facebook page). In order to assess ascertainment bias in the SCT group three subgroups were identified: (A) 'active

prospective follow-up' included families that were actively followed after prenatal diagnosis (51.5% of the SCT group), (B) 'Information seeking parents' included families who enrolled in the study because they wanted more information about SCT, but did not have specific concerns about the development of their child (27.2% of the SCT group), and (C) 'Clinically referred cases' included families who enrolled after receiving professional help because of specific concerns about the development of their child (21.4% of the SCT group). Non-clinical controls were recruited from the western part of the Netherlands. In collaboration with public sites, such as public daycare centers and public schools, and with the help of government institutions we had access to the civil registry. Via these public sites, information brochures were distributed to parents with children of eligible age. If parents were interested in the study, they were able to contact the researchers to receive further information about the study and to discuss enrollment.

For all participants, both the child and parent had to speak Dutch or English. Exclusion criteria included a history of traumatic brain injury, severely impaired hearing or sight, neurological illnesses, or colorblindness. Specific for the non-clinical control group, children with a previous diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013) were excluded. SCT was defined by trisomy in at least 80% of the cells and was confirmed by standard karyotyping. For ethical reasons, genetic screening was not performed in the control group. As the prevalence of SCT ranges from 1:650-1:1000, the risk of inclusion of a child with SCT in the control group was considered minimal and acceptable.

Background Information of Participants

Global intellectual functioning (GIF) was assessed with the Bayley Scales of Infant and Toddler Development (N_{SCT} = 34; $N_{control}$ = 31; Bayley, 2006) in the one-year-olds, and the short-version of the Wechsler Preschool and Primary Scale of Intelligence third edition (N_{SCT} = 61; $N_{control}$ = 71; WPPSI; Wechsler, 2002) or the Wechsler Nonverbal Scale of Ability (N_{SCT} = 2; Wechsler & Naglieri, 2006) in children aged three years or older. GIF scores for six children in the SCT group were missing. There was a significant difference in average full-scale intelligence scores between the SCT and control group, $t(197) = -3.70$, $p < .001$, $d = .53$. Although both groups on average scored within the average range, the SCT group scored lower ($M = 97.45$, $SD = 17.01$) than the control group ($M = 105.69$, 14.34). For the children assessed with the WPPSI, non-verbal reasoning scores were also available; children in the SCT group scored significantly lower ($N = 62$, $M = 96.48$, $SD = 17.16$) than the control group ($N = 71$, $M = 106.35$, $SD = 14.56$, $p < .001$, $d = .62$).

As a marker for socioeconomic status (SES), parents were asked to indicate the highest level of education they had received. Data was collected for both caregivers. To be able to compare data from participants from all countries, parental education was converted to a global scale with the criteria of Hollingshead (Hollingshead, 1975). The Hollingshead scale ranges from 0 (no formal education) to 7 (graduate/professional training). The highest level of education according to the Hollingshead criterion was then averaged for both caregivers. If no second caregiver was present (3.9% of the participants), the level of education for only one parent was used. SES for one child in the SCT group was unknown. There was a significant

difference in average SES between the SCT and the control group, $t(176.70) = 2.99$, $p = .003$, $d = .42$. On average, the SES of the SCT group was higher ($M = 5.93$, $SD = .94$) than the SES of the control group ($M = 5.43$, $SD = 1.40$).

Lastly, we looked at average parental age, where age of both caregivers was averaged. Parental age for one child in the SCT group was missing. Parental age was significantly higher in the SCT group ($M = 39.21$, $SD = 4.99$) than in the control group ($M = 36.02$, $SD = 5.19$), $t(202) = 4.46$, $p < .001$ $d = .63$.

As there were significant differences between the SCT and control group on global intellectual functioning, SES, and average parental age, correlations were calculated between these variables and all outcome measures for each age group and for the SCT and control group separately. All correlations can be found in the supplementary materials.

Age Groups

To test for age dependent differences, participants were divided into the following age groups: (1) the 1-year-old group (aged 11-23 months; $N_{SCT} = 35$, $N_{controls} = 31$), (2) the 3-4-year-old group (aged 35-59 months; $N_{SCT} = 42$, $N_{controls} = 45$), and (3) the 5-6-year-old group (aged 60-83 months; $N_{SCT} = 26$, $N_{controls} = 26$). The number of included children in the SCT group and descriptives per age group can be found in Table 2. The ratio of SCT karyotypes was assessed across age groups, there were no significant differences ($p = .093$) indicating that the distribution of karyotypes was similar in each age group.

Procedure

This study was approved by the Ethical Committee of Leiden University Medical Center, the Netherlands, and the Colorado Multiple Institutional Review Board (COMIRB) in Colorado, USA. After providing a description of the study to the parent(s) of the child, written informed consent according to the declaration of Helsinki was obtained.

Assessment took place at various sites (Colorado USA, the Netherlands, Belgium) either in a quiet room at the university or at home. To standardize the testing environment, the testing set-up and research protocols were identical on all sites. Researchers from Leiden University were responsible for project and data-management (i.e., training and supervision of researchers, processing and scoring of data).

Due to the inclusion of participants from various sites, tasks and questionnaires were administered in either Dutch or English. With the exception of one task, all tasks and questionnaires were available in both languages. The Dutch and English versions of the tasks and questionnaires are very similar, with sufficient psychometric properties, and can be used interchangeably. Both versions come with language-specific norms based on population samples. For one questionnaire, the number of items differed between the Dutch and English versions; adjustments in the scores were made when applicable. As the task to assess phonological processing skills was not available in Dutch, this task was administered in the USA group only. All tests and questionnaires were administered and interpreted according to the standardized procedure as specified in the instrument's manual.

Table 2. SCT versus controls per age group

	1-year-olds			3-4-year-olds			5-6-year olds			SCT Comparisons by age groups
	SCT	**Control**	***p***	**SCT**	**Control**	***p***	**SCT**	**Control**	***p***	
Total *N*	35	31		42	45		26	26		
Age	1.39 (.36)	1.53 (.28)	.073	3.87 (.60)	3.87 (.60)	.981	5.90 (.59)	5.62 (.40)	.044	
GIF	99.32 (13.41)	99.71 (13.98)	.910	102.36 (19.15)	105.73 (14.70)	.364	86.83 (13.44)	112.77 (10.96)	<.001	1 = 3-4 > 5-6
Verbal IQ[a]				102.74 (18.67)	106.82 (17.43)	.306	85.83 (12.30)	114.58 (16.10)	<.001	
Nonverbal IQ[b]				100.26 (17.60)	104.02 (14.82)	.290	90.09 (14.62)	110.38 (13.44)	<.001	
Ratio Karyotypes XXX/XXY/XYY	6/21/8			13/20/9			13/8/5			XXX = XXY = XYY

Note: scores represent Means (SD)
SCT = Sex Chromosome Trisomy; SCT comparisons by age group = comparison of outcome in 1-year-olds with SCT versus 3-4-year-olds with SCT versus 5–6-year-olds with SCT; GIF = level of global intellectual functioning
[a] Verbal IQ was missing for 4 SCT children in the 3–4-year-old group and 3 SCT children in the 5–6-year-old group
[b] Nonverbal IQ was missing for 3 SCT children in the 3–4-year-old group and 3 SCT children in the 5–6-year-old group

Instruments

Early Non-Verbal Communication and Early Vocabulary

Within the youngest age group (1-year-olds), parents were asked to complete the age-appropriate version of the MacArthur-Bates Communicative Development Inventories (CDI), either in English (Fenson et al., 2007) or in Dutch (Zink & Lejaegere, 2014). For children aged 11-16 months, parents filled out the Words and Gestures (CDI W&G) form. For children aged 17-23 months, parents filled out the Words and Sentences (CDI W&S) form. The CDI was completed by the primary caregiving parent (92.1% mother) of the child.

Words and Gestures – Early Non-Verbal Communication.

Early forms of communication for children aged 11-16 months were assessed with the CDI Words and Gestures part II: Actions and gestures, which consists of five subsections. Subsections A and B together measure 'early gestures', and address questions regarding the first communicative gestures as a measure of the onset of intentional communication (subsection A) and games and routines as a measure of the early social interactive basis for communicative development (subsection B). Subsections C trough E measure 'later gestures', and address questions regarding actions with objects and imitating other adult actions as a measure of understanding of the world of objects and the use of things (subsections C and D) and pretending to be a parent as a measure of true symbolic gestures (subsection E). Depending on the form used (USA versus Dutch form respectively), 17/18 early gestures and 45/48 later gestures were assessed.

Words and Gestures - Early Vocabulary.

Early vocabulary of children aged 11-16 months was assessed with the CDI Words and Gestures part I – subsection D: Vocabulary checklist. Within the vocabulary checklist, parents can indicate which of the words a child understands (receptive early vocabulary) and which of the words a child understands and says (expressive early vocabulary). The number of items included in the vocabulary checklist depends on the used form, with 396 items in the USA form, and 434 items in the Dutch form.

Words and Sentences – Early Vocabulary.

Early vocabulary of children aged 17-23 months was assessed with the CDI Words and Sentences part I – subsection A: Vocabulary checklist. The administration of the Dutch version of the vocabulary checklist is similar to the CDI W&G vocabulary, with a total number of 702 items. The USA version of the checklist, however, only requires parents to indicate which of the words a child says (expressive early vocabulary), with a total of 680 items.

Semantic Language Skills

Semantic language skills were assessed with the Bayley Scales of Infant and Toddler Development (Bayley, 2006) in the 1-year-olds, and with the Clinical Evaluation of Language Fundamentals Preschool (CELF-P; Wiig et al., 2004, 2012) and the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997, 2005) in the 3-6-year-olds.

One-year-olds.

The Bayley Scales were used as an indicator for the development of children aged 1-42 months in five developmental domains. For this study, only the language scale was used. The Bayley Language Scale consists of separate subtests for receptive and expressive communication. The receptive communication subtest assesses pre-verbal behavior, ability to identify objects and

pictures, and understanding of verbal messages. The expressive communication subtest assesses pre-verbal communication, ability to name objects and pictures, and the ability to use multiple-word sentences.

Three-to-six-year-olds.

The CELF-P was used to assess several elements of language in children aged 3-7 years. For this study, the CELF-P subtest Expressive Vocabulary was used. This subtest assesses the ability to label people, objects, and actions based on colored images. Higher scores indicate better expressive vocabulary skills.

The PPVT was used to assess receptive vocabulary in individuals aged 2-90+ years. This test measures listening comprehension of spoken words. For each item, the participant is shown four black and white pictures, and the participant has to identify the picture that illustrates the stimulus word that is orally presented by the researcher. Higher scores indicate better receptive vocabulary skills.

Syntax and Phonological Processing

Within the 3-4- and 5–6-year-old children, the subtest Sentence Structure from the CELF-P was used as an indication of syntactic development. This subtest assesses the ability to interpret sentences that increase in length and structural complexity. The child was presented four colored pictures on one page and had to select the picture that illustrated the sentence that was orally presented by the researcher. Higher scores indicate better syntactic understanding.

In the USA 3-4- and 5–6-year-old groups, phonological processing skills were assessed with the NEPSY-II phonological processing subtest (Korkman et al., 2007a, 2007b). In the 3–4-year-old group, phonological processing was assessed using word segment recognition. This subtest assesses a child's ability to identify a word when given an orally presented word segment (e.g., "-og" for dog). In the 5–6-year-old group, elision at the syllable and phoneme level was also used in addition to the word segment recognition task.

Statistical Analyses

Raw Scores, Clinical Risk Assessment, and Z-scores

Three types of scores were used. First, raw scores were used to compare the children in the SCT versus the control groups. Raw scores (scores unadjusted for age) were preferred over standardized scores to examine the relation between age and language skills for each age group separately. Secondly, raw scores were converted into percentile scores based on age and country specific norms. Percentile scores were then divided into categories to assess variability of scores within the SCT group based on the psychometric conversion table for neuropsychological tests (Lezak et al., 2004). This resulted in the following seven categories: 1) Severely impaired (percentile score of 1.99 or lower), 2) mildly impaired (percentile scores between 2-8), 3) low average (percentile scores between 9-24), 4) average (percentile scores between 25-75), 5) high average (percentile scores between 76-91), 6) superior (percentile scores between 92-97), and 7) very superior (percentile score of 98 or higher). In addition, clinical risk was assessed; when a child scored below the 16th percentile (i.e., 1 *SD* below mean), this child was considered as having 'language difficulties'. Finally, standard and scaled scores were converted into z-scores

with the same psychometric conversion table in order to compare outcomes on language domains independent of type of (age appropriate) test.

Analyses

Karyotype and boy/girl specific outcomes were compared with nonparametric Kruskal Wallis tests or ANCOVA in case of age differences between groups. SCT versus control group differences were analyzed with one-way-between subjects ANOVA, with the language scores as dependent variables and research group as independent variable. ANOVA was run for each age group separately. To assess the impact of SCT specific characteristics (i.e., time of diagnosis, ascertainment bias, research site), one-way ANOVA was used as well. When applicable, post-hoc analyses were used to identify significant group effects. Effect sizes were calculated with Cohen's d when applicable, where $d = \frac{M1-M2}{\sqrt{\frac{(n1-1)SD1^2+(n2-1)SD2^2}{n1+n2-2}}}$. Clinical risk assessment was done with descriptive frequencies and as an indication of effect size, odds ratio was calculated.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 25. Level of significance was set at $p \leq .05$, two-tailed. Analyses were run initially without covariates, and to account for differences in nonverbal abilities run again in the 3-4- and 5–6-year-olds with nonverbal IQ as covariate. Due to the number of statistical analyses, a correction of alpha (i.e., the Benjamini-Hochberg Procedure) was conducted to control the false discovery rate.

Results

Karyotype Specific Language Outcomes

First, as boys and girls may develop language at a different pace (Eriksson et al., 2012), we compared language outcomes of boys and girls in our control sample for all language outcomes with the nonparametric Kruskal Wallis test due to sample sizes. Within the one-year-old control group, there was a significant age difference between boys and girls, with the average age slightly higher in the girls. For that reason, group comparisons in this group were analyzed with ANCOVA with age as covariate. Within the 3-4 and 5-6-year-olds, age was not statistically different between boys and girls. For all of the included language outcomes, results were not statistically different between boys and girls (*p* ranging from .118 to .998). For that reason, we did not expect sex-differences within our SCT group, and karyotype specific outcomes (i.e., XXX, XXY, and XYY) were compared.

Explorative, karyotype specific outcomes for the language domains were assessed. First, with ANOVA, receptive semantic and expressive semantic language skills were compared. Z-scores were used to compare scores regardless of used instrument. No significant differences between the three karyotypes were found for receptive (p =.493) or expressive semantic language skills (p = .106). Next, the nonparametric Kruskal Wallis test was used to assess karyotype specific outcomes within each age group. Average age was compared between the three karyotypes in each age group and no significant differences were found. The nonparametric Kruskal Wallis test did not yield significant differences for the language outcomes (*p* ranging from .118 to .966). For each of the language outcomes, number of included

children per karyotype, average outcomes, and the results of the ANOVA and Kruskal Wallis tests are shown in Table 3.

For each karyotype separately, clinical classification was conducted by calculating the percentage of children with 'language difficulties' (i.e., a score at or below the 16th percentile). Due to the small sample size for some karyotypes in the age groups, age groups were collapsed in this analysis. For girls with an extra X chromosome, 23.3% (7/30) had difficulties with receptive semantic skills, and 35.5% (11/31) had difficulties with expressive semantic skills. For boys with an extra X chromosome, 14.3% (7/49) had difficulties with receptive semantic skills, and 18.4% (9/49) had difficulties with expressive semantic skills. For boys with an extra Y chromosome, 20.0% (4/20) had difficulties with receptive semantic skills, and 36.8% (7/19) had difficulties with expressive semantic skills. A visualization of results can be found in Figure 1.

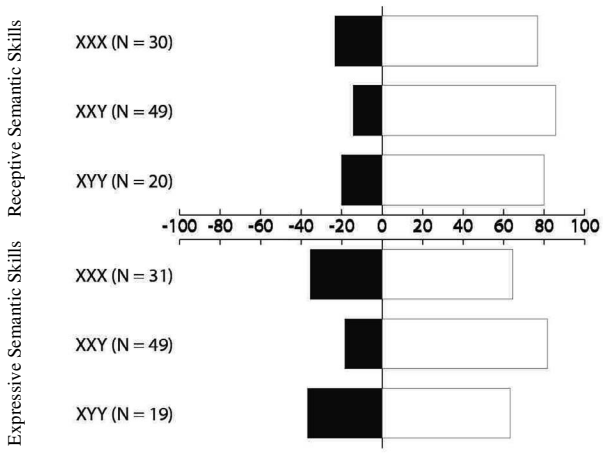

Figure 1. Percentage of SCT children with language difficulties (i.e., scores at or below the 16th percentile) on receptive and expressive semantic skills per karyotype

As there were no significant differences between the SCT karyotypes on the language outcomes, the three SCT karyotypes were collapsed into one group (SCT group) for subsequent analyses.

Table 3. Karyotype specific outcomes on the language domain: Overall and per age group

		N (XXX/XXY/XYY)	XXX	XXY	XYY	*p*
Overall	Receptive semantic skills	30/49/20	-.09 (.91)	.02 (.94)	-.28 (1.01)	.493
	Expressive semantic skills	31/49/19	-.55 (1.10)	-.21 (.84)	-.72 (1.07)	.106
1-year-olds	Early receptive vocabulary	4/12/5	36.25 (36.90)	37.25 (24.17)	43.60 (32.07)	.966
	Early expressive vocabulary	6/19/8	22.67 (16.84)	40.58 (20.66)	34.00 (22.59)	.167
	Receptive semantic skills	6/21/7	18.50 (5.24)	17.48 (5.42)	18.43 (4.39)	.694
	Expressive semantic skills	6/21/7	18.67 (7.29)	18.76 (5.64)	18.71 (5.96)	.757
3-4-year-olds	Receptive semantic skills	12/20/9	56.75 (16.67)	54.35 (16.39)	46.44 (17.66)	.401
	Expressive semantic skills	12/20/8	16.58 (7.69)	15.45 (5.88)	11.88 (9.99)	.490
	Syntax	12/20/8	12.75 (5.23)	10.70 (4.44)	11.13 (4.91)	.407
5-6-year-olds	Receptive semantic skills	12/8/4	72.75 (10.67)	86.38 (13.15)	75.00 (18.49)	.312
	Expressive semantic skills	13/8/4	24.54 (6.28)	28.75 (7.07)	27.75 (7.14)	.118
	Syntax	12/8/4	15.33 (4.44)	17.50 (2.67)	15.25 (4.27)	.661

Scores represent means (SD) based on raw scores. p-values for the age groups represent outcomes from the nonparametric Kruskal-Wallis test based on mean ranks.

Language Difficulties at Different Developmental Stages

One-year-old Children

There was missing data for one or more of the assessments for three children in the SCT group and one child in the control group. Mean results and effect sizes per language domain can be found in Table 4.

Early Receptive and Expressive Vocabulary

A one-way between-subjects ANOVA compared the mean raw scores of receptive and expressive vocabulary for children with SCT and controls. For receptive vocabulary, there was a significant difference between children with and without SCT, Welch's $F(1, 48.76) = 18.12$, $p < .001$. On average, children with SCT had a smaller receptive vocabulary than the control group. For expressive vocabulary, there was also a significant difference between children with and without SCT, Welch's $F(1, 35.13) = 8.60$, $p = .006$. On average, children with SCT had a smaller expressive vocabulary than the control group. Effect sizes for both receptive and expressive vocabulary for one-year-old children indicate large deviations.

Semantic Language Skills

A one-way between-subjects ANOVA compared the mean raw scores of receptive and expressive semantic skills for children with SCT and controls. For receptive semantic skills, there was a significant difference between the SCT and the control group, $F(1,63) = 15.02$, $p < .001$. On average, children with SCT had lower receptive semantic skills than controls. For expressive semantic skills, there was also a significant difference between the SCT and the control group, $F(1,63) = 10.72$, $p = .002$. On average, children with SCT had lower expressive semantic skills than controls. Effect sizes for both receptive and expressive semantic skills indicate large deviations.

Three-and-four-year-old Children

There was missing data for one or more of the assessments for three children in the SCT group and two children in the control group. Mean results and effect sizes per language domain can be found in Table 4.

Semantic Language Skills

A one-way between-subjects ANOVA compared the mean raw scores of receptive and expressive semantic skills for children with SCT and controls. For receptive semantic skills, there was no significant difference between the SCT and the control group, $F(1,82) = 2.34$, $p = .130$. Children with SCT on average performed similarly to controls. For expressive semantic skills, there was a significant difference between the SCT and the control group, $F(1,82) = 31.01$, $p < .001$. On average children with SCT had significantly lower expressive semantic scores than controls. Effect sizes for expressive semantic skills indicate large deviations.

Syntax

A one-way between subjects ANOVA compared the mean raw scores of syntactic language skills for children with SCT and controls. There was no significant difference between the SCT and control group, $F(1,82) = 2.60$, $p = .111$. Children with SCT in this age group had similar syntactic skills as controls.

Five-and-six-year-old Children

There was missing data for one or more of the assessments for two children in the SCT group. Mean results and effect sizes per language domain can be found in Table 4.

Semantic Language Skills

A one-way between-subjects ANOVA compared the mean raw scores of receptive and expressive semantic skills for children with SCT and controls. For receptive semantic skills, there was a significant difference between the SCT and the control group, Welch's $F(1,32.45) = 14.45$, $p = .001$. On average, the children with SCT had lower receptive semantic skills than the controls. For expressive semantic skills, there was also a significant difference between the SCT and the control group, Welch's $F(1,34.18) = 24.89$, $p < .001$. On average, children with SCT had significantly lower expressive semantic scores than controls. Effect sizes for both receptive and expressive semantic skills indicate large deviations.

Syntax

A one-way between subjects ANOVA compared the mean raw scores of syntactic language skills for children with SCT and controls. There was a significant difference between the SCT and control group, Welch's $F(1,31.87) = 20.40$, $p < .001$. Children with SCT had lower syntactic language skills than the controls. Effect sizes indicate large deviations.

The Effect of Non-Verbal Intelligence on Language Outcomes and Corrective Analyses

To assess the effect of non-verbal intelligence on language outcomes, all statistical analyses in the 3-4- and 5–6-year-olds were run with non-verbal intelligence as covariate. For all analyses, the pattern of findings was the same as without the correction for nonverbal intelligence. This indicates that the differences between children with and without SCT on language outcomes remain significant, irrespective whether or not a deficit in nonverbal IQ was present.

Due to the multiple statistical analyses, a Benjamini-Hochberg Procedure was run to control the false discovery rate. Results after the procedure followed the same pattern of findings, indicating that significant results represent true findings rather than false discoveries.

Table 4. Mean results and effect sizes for each language domain per age group: SCT versus control

	1-year-olds			3-4-year-olds			5-6-year-olds		
	SCT	**Cont**	***d***	**SCT**	**Cont**	***d***	**SCT**	**Cont**	***d***
Early receptive vocabulary	19.83 (18.81)	48.42 (29.10)	1.13***	N/A	N/A		N/A	N/A	
Early expressive vocabulary	5.07 (7.87)	18.04 (23.03)	.77**	N/A	N/A		N/A	N/A	
Receptive semantic skills	17.85 (5.07)	22.32 (4.13)	.96***	53.32 (16.77)	58.37 (13.42)	.33	77.67 (13.85)	89.50 (6.64)	1.10***
Expressive semantic skills	18.74 (5.81)	23.32 (5.45)	.81**	15.08 (7.37)	23.27 (6.11)	1.22***	26.40 (6.68)	33.77 (3.20)	1.42***
Syntax	N/A	N/A		11.40 (4.74)	13.14 (5.10)	.35	16.04 (3.88)	19.96 (1.80)	1.31***

Note: scores represent Means (SD)
SCT = Sex Chromosome Trisomy; Cont = controls; N/A = not applicable – test was not administered in this age group
Significance: * $p < .05$; ** $p < .01$; *** $p < .001$
Cohen's *d* effect size SCT versus controls

Affected Language Domains in Children with SCT: Variability and Clinical Classifications

When applicable, raw data were converted to percentile scores and classified based on a psychometric conversion table. Children who scored below the 16th percentile were considered as having 'language difficulties'; the percentage of children on each of the language outcomes are described below. Table 5 displays the variability in outcomes (i.e., percentage of children per clinical classification), the percentages of children with 'language difficulties' and the odds ratio (i.e., the change of having language difficulties in the SCT group compared to the control group) for each language domain. A visual representation of the percentage of children with language difficulties can be found in Figure 2.

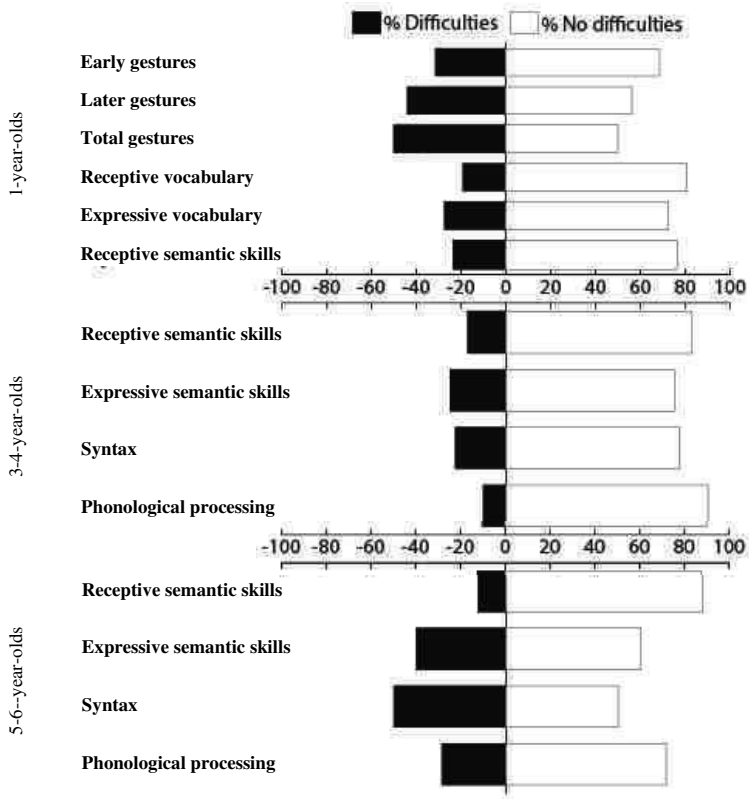

Figure 2. Visual representation of language difficulties per age group for SCT children only. The dark bars represent the percentage of children with scores at or below the 16th percentile on each of the language domains.

Early Non-Verbal Communication Skills: Actions and Gestures

Within the youngest age group (11-15 months; M_{age} = 12.6 months, SD = 1.22 months) of children with SCT (N = 16) the CDI questionnaire asks parents about the number of gestures their child uses. Already with these earliest forms of communication, up to half of the children with SCT had difficulties.

Early Receptive and Expressive Vocabulary

Parents of all one-year-olds with SCT were asked to indicate how many words their child understood and/or produced ($N_{understood}$ = 21; $N_{produced}$ = 33). Classification of these results show that already at this age, receptive and/or expressive vocabulary skills can be (severely) impaired in children with SCT when their performance is compared to children from the same country and the same age. Within this sample of one-year-old children with SCT, 19.0% had difficulties with understanding words (or receptive vocabulary), and 27.3% with producing words (or expressive vocabulary).

Semantic Language Skills

Receptive and expressive semantic skills were assessed in children of all ages ($N_{receptive}$ = 99; $N_{expressive}$ = 99). Overall, for receptive semantic skills, 18.2% of the children performed below what is expected at their age (N = 23) and 27.3% of the children had expressive semantic skills below what is expected (N = 27).

The large sample size allowed clinical classification per age group, which showed that in one-year-old-children, 23.5% had difficulties with receptive semantic skills (N = 34), and 20.6% had difficulties with expressive semantic skills (N = 34). In the 3-4-year-old group, receptive semantic skills were assessed in 41 children and expressive semantic skills in 40 children. In this group of 3-4-year-olds, 17.1% had difficulties with receptive semantic skills, and 25.0% had difficulties with expressive semantic skills. Finally, in the 5-6-year-old group, receptive semantic skills were assessed in 24 children and expressive semantic skills were assessed in 25 children. In this group of 5-6-year-olds, 12.5% of the children had difficulties with receptive semantic skills and 40.0% had difficulties with expressive semantic skills.

Syntax and Phonological Processing

As syntax and phonological processing were only assessed in 3-6-year-old children, age groups were collapsed for maximum statistical power. For syntactic development (N = 64), 32.8% of the children performed below what is expected at their age. Phonological processing skills were only assessed in the USA group, resulting in a smaller group of participants (N = 27). Overall, phonological processing skills appear to be affected in a smaller group of children with SCT.

Table 5. Clinical classification of children with language difficulties and variability per language domain for 1-6-year-old children with SCT

		Clinical Classification: Language Difficulties	Clinical Classification: Variability						
	N		Severely impaired	Mildly Impaired	Low Average	Average	High Average	Superior	Very Superior
Early gestures	16	31.3% OR = 7.17		6.3%	31.3%	31.3%	18.8%	12.5%	
Later gestures	16	43.8% OR = 1.94		6.3%	56.3%	31.3%	6.3%		
Total gestures	16	50.0% OR = 6.00		18.8%	31.3%	43.8%	6.3%		
Receptive vocabulary	21	19.0% OR = 2.12	4.8%	9.5%	28.6%	52.4%	4.8%		
Expressive vocabulary	33	27.3% OR = 1.88	6.1%	6.1%	18.2%	69.7%			
Receptive semantic skills	99	18.2% OR = 5.33	1.0%	8.1%	14.1%	52.5%	18.2%	6.1%	
Expressive semantic skills	99	27.3% OR = 5.04	5.1%	8.1%	14.1%	65.7%	5.1%		2.0%
Syntax	64	32.8% OR = 2.93	3.1%	15.6%	14.1%	56.3%	9.4%		1.6%
Phonological processing	27	14.8% OR = N/A[a]			14.8%	77.8%			7.4%

Note: Language difficulties: Children who scored at or below the 16th percentile; OR = odds ratio

[a] There was no control group available for the Phonological processing task; the OR could not be calculated.

Semantic Language Outcomes: Impact of SCT Characteristics

The impact of time of diagnosis (prenatal versus postnatal), ascertainment bias (prospective follow up, information seeking, and clinically referred), and research site on language outcomes was assessed with ANOVA. Only measures for receptive semantic and expressive semantic skills were included, as these outcomes were available for participants of all ages. To allow for comparisons regardless of used instrument, standardized and scaled scores were converted into z-scores based on the psychometric table. There were no significant differences in either receptive or expressive semantic outcomes for prenatal versus postnatal diagnosis, for ascertainment bias, or for research site. Results can be found in Table 6.

Table 6. SCT characteristics and average scores for expressive and semantic language skills

	Time of Diagnosis			Ascertainment Bias[a]				Recruitment Site		
	Prenatal	**Postnatal**	***p***	**A**	**B**	**C**	***p***	**USA**	**NL/BE**	***p***
N	67	31		51	26	21		56/55	43/44	
Receptive semantic skills	-.05	-.19	.513	-.09	-.06	-.15	.941	-.17	.04	.280
	(.94)	(.90)		(.94)	(.95)	(.92)		(.97)	(.91)	
Expressive semantic skills	-.33	-.60	.205	-.42	-.31	-.54	.731	-.43	-.39	.854
	(1.03)	(.89)		(.86)	(1.02)	(1.26)		(1.02)	(.96)	

Note: scores represent Means (SD)

[a] Ascertainment bias: A = Active prospective follow up; B = Information seeking parents; C = Clinically referred cases

Discussion

The goal of this cross-sectional study was to describe the language profile of a large group of young children with SCT at an age when language is undergoing rapid growth, and by assessing multiple language domains to pinpoint on which of the language outcomes children experience difficulties. For that reason, this study aimed to answer the following key questions: First, to identify the language profiles in children with SCT at different developmental stages within the 1-to-6-year age range. Second, to identify the proportion of children with difficulties in language development and to describe the variability of language development within the SCT group. Finally, in addition to these key questions, this study aimed to evaluate factors that could impact language outcomes (i.e., SCT karyotype, time of diagnosis, ascertainment bias, and research site).

Several factors that could affect language outcomes were assessed: SCT karyotypes, time of diagnosis, ascertainment bias, and research site. Regarding karyotype specific outcomes, we first compared receptive and expressive semantic skills between XXX, XXY, and XYY for children of all ages. In line with earlier studies, our results indicated that there were no significant differences between the three SCT karyotypes on these two language outcomes (e.g., Bishop et al., 2018; Lee et al., 2012). Next, explorative, we looked at karyotype specific differences within each age group and did not find differences between the three groups on the included language outcomes. However, due to small sample sizes, results should be interpreted with caution. In addition – when controlling for age-dependent factors – we did not find differences in semantic outcomes between children with a prenatal diagnosis or postnatal diagnosis, nor were outcomes related to ascertainment bias (i.e., the way participants enrolled in the study), or research site. Overall, it appears that language outcomes are very robust within the SCT group. As we did not find evidence for significant differences between the three SCT karyotypes and as previous MRI studies have implied homologous effects of an extra X or Y

chromosome on development of the brain (Raznahan et al., 2016), we considered the children with SCT as one group for further analyses to explore age dependent effects in more detail.

With regard to the first aim, the results indicated that children with SCT on average have poorer language skills than children without SCT. In one-year-old children, children with SCT produced and understood fewer words than their peers without SCT according to parent report. Within this same age group, poorer receptive and expressive semantic skills were also found with neuropsychological assessment. When ranking the effect sizes within the one-year-old group, the largest deviations from the control group were found for early receptive vocabulary. In three-to-four-year-old children, children with SCT on average had poorer expressive semantic skills than their peers without SCT. Within this age group, however, we found similar receptive semantic skills and (receptive) syntactic language skills in children with SCT and children without SCT. In the five-to-six-year-olds, children with SCT had poorer receptive and expressive semantic skills as well as poorer (receptive) syntactic skills than their peers without SCT. When ranking the effect sizes for this age group, the largest deviations from the control group were found for expressive semantic skills. There was a slight difference in the average age and age distribution (not reported) between the SCT and control group in the 5-6-year-old group, with relatively more older children in the SCT group. As this difference was not in favor of the SCT children (i.e., due to the higher age, higher scores could have been expected), the results presented here might be a slight underestimation.

Collectively, these results imply that the increased risk for language problems starts at a very early age, and that poorer skills compared to children without SCT are a robust finding across developmental stages and the various language domains. Such early language difficulties in the SCT population fit with the idea that language impairments are anchored in early brain development. Studies have shown that both the X and Y chromosomes contain genes that are important for neural development and related cognitive functions (Lenroot et al., 2014; Raznahan et al., 2016). Neuroimaging studies have been conducted to research the consequences of the extra sex chromosome on both the structure and the functioning of the brain. Although studies that provide evidence of a direct link between structural differences and language outcomes in individuals with SCT are lacking (for a review see Skakkebaek et al., 2020), structural differences between children with and without SCT have been found in brain regions that are anatomically consistent with areas that are important for language and/or play a role in language-based learning difficulties (Bryant et al., 2012; Giedd et al., 2007; Lenroot et al., 2014). Only a handful of studies have used functional neuroimaging to test differences in brain activation during a language task, and most of these studies focused on differences in language lateralization (van Rijn et al., 2008; Wilson & Bishop, 2018). Results of these studies are somewhat mixed; a study of school-aged children did not find differences in language lateralization (Wilson & Bishop, 2018), whereas a study of adults did find differences (van Rijn et al., 2008). Given that compromised language development is anchored very early in development, longitudinal studies are needed to model to what degree early markers of language difficulties predict cognitive and behavioral outcomes, as well as risk for psychopathology later in life.

Second, outcomes were categorized according to clinical guidelines and compared to performance expected at each child's chronological age. Results showed that there is much variability within the SCT group. While some children score in the average or above average range, a group of children with SCT performs below what is expected for their age. Based on the classification of language difficulties (i.e., children who scored at or below the 16th percentile), children in the one-year-old-group showed increased risk for difficulties not only with spoken language, but also with nonverbal communication, such as using gestures for intentional communication and imitating adult actions. The percentage of children that experiences difficulties ranged from 31.3 to 50 percent. In addition to these difficulties with early nonverbal communication, a group of 1-year-old children with SCT has difficulties with early receptive (19.4%) and/or expressive (27.3%) vocabulary skills. Regarding semantics, 23.5% of the one-year-olds children had difficulties with receptive semantic skills, and 20.6% of the children had difficulties with expressive semantic skills. Within the 3–4-year-old-group, we found that 17.1% of our group experienced difficulties with receptive semantic skills and a quarter of the children with expressive semantic skills. Within the 5-6-year-olds, this was the case for 12.5% of the children with receptive semantic skills, and for expressive semantic skills, 40.0% of the children experienced difficulties. Odds ratio indicated that the risk of language difficulties was 2-7 times higher in the SCT group as compared to the control group, depending on the language function. Collectively, these results show that a large group of children with SCT already has a disadvantage from an early age. When ranking the odds ratio, the SCT group, compared to the control group, had the highest odds for clinical scores on the domain of early nonverbal communication, followed by receptive semantics and expressive semantics. We speculate that these domains are affected the most in the SCT group, taking into account that sample sizes differ between language outcomes. Although this is a cross-sectional sample, these results indicate that difficulties on some domains may become more prominent with increasing age, warranting early support and preventive intervention.

To our knowledge, this is one of the first studies that included a large group (N = 103) of children with SCT at a very young age when language is developing rapidly, and that studied several language domains. There are three other recent studies focusing on language skills in very young children with SCT. These studies include groups of children that participated in a clinical monitoring program in Italy. Similar to our results, these three studies also indicated that compromised language is evident in very young children with SCT. Regarding early communication skills, in contrast to our results, Zampini et al. (2018) found no differences in the number of gestures used by 18-month-old boys with XXY (N = 13) compared to typically developing boys. A second study by this group, with 24-month-old children (8 XXY and 7 XXX) however, found that children with an extra X chromosome used *more* gestures than children without the extra chromosome (Zampini et al., 2017). As the children included in this study were older (24 months of age), it is possible that as age increases, children start to compensate for their verbal difficulties by using more gestures, a finding that has also been established in other clinical populations, such as children with specific language impairment, down syndrome, or autism (Capone & McGregor, 2004). It should also be noted that the findings by Zampini et al. (2017; 2018) are based on observed spontaneous communicative acts during an unstructured play session, whereas our findings are based on parent report, therefore

the studied gestures may differ between studies. Early receptive and expressive vocabulary has also been assessed by the research group of Zampini and colleagues. In a group of 8-month-old children (9 XXY, 10 XXX, 7 XYY), no significant differences in receptive vocabulary were found between children with and without SCT (Zampini et al., 2020). Expressive vocabulary was assessed in the 13 boys with XXY at 18 months and the boys and girls with an extra X at 24 months. These results show that compared to typically developing peers, children with an extra X chromosome at 18 and 24 months have significantly lower expressive vocabularies (Zampini et al., 2017; 2018). Similar to our findings, Zampini et al (2017, 2020) found no differences between the SCT groups. Finally, differences in outcomes between the Italian studies and the current study could also be due to differences in recruitment (i.e., a clinical sample versus a research sample).

With regard to studies assessing receptive and expressive vocabulary skills in broader age groups (up to 18 years), studies report mixed findings. One study with 4–18-year-old XXY boys found age appropriate receptive and expressive vocabulary scores when comparing the boys to the norming sample (Ross et al., 2008). A second study by the same research group with 4–18-year-old boys (XXY and XYY) compared outcomes to typically developing boys (Ross et al., 2009). For both the XXY and the XYY group separately, authors reported lower receptive and expressive vocabulary scores compared to the typically developing controls. In addition, the authors compared outcomes between XXY and XYY boys. For expressive vocabulary, no differences were found, whereas for receptive vocabulary, the XYY boys had worse outcomes than the XXY boys.

With regard to studies that included assessments of semantics, syntax, and phonological processing in children with SCT up to 18 years generally show impairments on these language domains. A study by St John et al. (2019) similarly to our findings, reported lower overall receptive and expressive language skills in a group of boys ($N = 22$) with XXY aged 1-17 years. In addition, Ross et al. (2008) found that 4–18-year-old boys with XXY ($N = 50$), performed below age expectations compared to the norming sample on tests assessing semantic and syntactic language skills. In addition, when comparing the younger boys (4-10-year-olds) with the older boys (10-18-year-olds), the authors found significantly more problems in the older boys. Similar to our cross-sectional findings, these findings could imply that language problems become more substantial over time. Lastly, a study by Ross et al. (2009) showed impaired semantic and syntactic skills in boys with XXY ($N = 93$, aged 4-18 years) and XYY ($N = 21$, aged 4-14;4 years), with no differences in performance between these two groups. Reported outcomes for phonological processing skills are mixed, with some studies reporting impairments (e.g., Ross et al., 2009), whereas other studies report age-appropriate phonological processing skills (e.g., Ross et al., 2008), similar to our findings. As phonological processing has been shown in many studies to be a predictor of later literacy skills, and there is a large number of children with SCT to have later reading problems, it is important to learn more about the phonological development in very young children with SCT and to identify whether targeting phonological processing early may decrease risk for later challenges.

In our study, depending on the studied language domain, we found rates of clinically relevant difficulties ranging from 12 to 50 percent. These percentages are lower than reported

percentages in other studies (for reviews see Boada et al., 2009; Leggett et al., 2010; Robinson et al., 1983). It is possible that the percentage found in the current study are representative for children at this young age, and that the percentage of children that experience difficulties depends on the included (age)group. Although not longitudinally studied, results of this study indicate that problems, especially with expressive language, could intensify over time. This phenomenon is also known as 'growing into a deficit' and occurs when a child stays behind on what is expected with increasing age, resulting in a growing deviation of performance compared to peers (Rourke et al., 1983). Another explanation could be the method to examine language development. This study included both parent reports and neuropsychological testing; it is possible that percentages vary across studies depending on the included measurements. Some studies included in the reviews (e.g., by Boada et al., 2009; Leggett et al., 2010; Robinson et al., 1983) for example included not only specific language measures, but also speech assessments, auditory processing skills, verbal intelligence, or school reports. Also, some studies included verbal academic skills (e.g., reading, writing, spelling), language-based learning problems such as dyslexia, or (only) reported the number of children that have received speech- or language therapy. This study included a young group of children, regardless of time of diagnosis or ascertainment bias, and from multiple research sites to represent the SCT population. This study used valid and reliable standardized assessment in addition to parent report to assess language outcomes and to identify the percentage of children with language difficulties. Our results stress the importance of early assessment of language performance. Already from a young age, there are children with SCT who fall behind age-expectations on various language domains. If the number of children who experience language difficulties increases with age, clinicians should closely monitor the language development of children with SCT and intervene early when needed.

From a clinical perspective, our results highlight the importance of monitoring language development in children with SCT very early in development, at the earliest stages of nonverbal communication. As our results show, large differences were found on nearly all language domains. This stresses the importance that not only expressive, but also receptive language skills should be assessed on a regular basis. Language affects every day functioning. If language skills are compromised, this could affect outcomes in other domains, including academic achievement, and quality of life. Current findings stress the need for screening and close monitoring of language development in this group of children from an early age onwards, for example during routine child-monitoring programs. Through early intervention, parents should be supported to stimulate the language development of their child, which is important for all children, but could possibly be even more crucial for children with SCT. When a child does not meet language milestones, we recommend standard neuropsychological screening, which should include nonverbal communication, as well as receptive and expressive language skills. With neuropsychological screening, children who are at-risk for suboptimal language development could be identified and the outcomes of the screening could serve as a guide for a tailored treatment and/or intervention plan (e.g., speech therapy). Finally, studies should evaluate to what degree existing intervention programs are beneficial for children with SCT, and if not, specific interventions tailored to the needs of children with SCT should be developed.

Although our study included a large group of young children with SCT, there were also limitations to this study accompanied by suggestions for future research. First, it is possible that by dividing the group into smaller subgroups based on age, power to detect clinically relevant differences may have been lost. Also, within this study we included children within three age groups (i.e., 1-year old, 3-4-year-old, and 5-6-year-old children. As language develops rapidly in early childhood, further exploration regarding age-specific language abilities within smaller age groups is warranted. Second, we have looked at karyotype specific differences on language outcomes for each age group separately. Due to the sample sizes, our methods were explorative. To gain more insight in language profiles for each karyotype, future studies should include large samples to study both age-specific and karyotype-specific outcomes. Third, we included children with XXY syndrome regardless of whether children had received testosterone supplements. To our knowledge, there is only one randomized controlled trial (RCT) that assessed the outcome of androgen treatment (oral oxandrolone) on cognitive functioning in children (Ross et al., 2017). Although the RCT by Ross et al (2017) reported no effect of early androgen treatment on language outcomes, a large group of children (49%) in the present study had received testosterone replacement therapy. More RCTs on the effect of testosterone replacement treatment on neurocognitive outcomes in young children with SCT are needed before conclusions about potential risks or benefits can be made. Fourth, it should be noted that some of the included children were unable to participate in one or more of the tasks; it cannot be precluded that reported results are slightly underestimated. However, the various ascertainment strategies and the lack of impact of ascertainment strategies on outcomes, contributes to the generalization of results to the population of *diagnosed* children with SCT. Fifth, although we were able to look at several aspects of language development, it is important to gain more insight into the overall neurocognitive profile of children with SCT, including the broader communication domain (i.e., pragmatic language abilities, or language in an academic setting), but also for example social cognitive abilities and executive functioning. Although we did not find differences between the three karyotypes on the included language outcomes in this study, it is possible that there are karyotype specific differences on other domains, a question that should be addressed in future studies. It should also be noted that we found lower average nonverbal IQ in our 5–6-year-olds with SCT compared to controls, a finding that was not observed in our 3–4-year-olds. When exploring the neurocognitive profile of children with SCT it is important to also take nonverbal IQ into account, as children with SCT may have a nonverbal deficit in addition to other neurocognitive difficulties. Another aspect that should be taken into account in future studies are environmental factors; factors that could possibly moderate outcomes. In our study for example, we found a difference in SES between the SCT and control group, in favor for the SCT group. Although we did not find substantial correlations between SES and language outcomes in either the control or SCT group, we cannot preclude that SES could indirectly impact other mediating factors, such as services received. Finally, as this was a cross-sectional study, our interpretation of age effects is based on different children, and language development over time should be assessed with longitudinal studies. Within these longitudinal studies, other possible confounding factors, such as familial learning difficulties and services received, should also be taken into account. Recently, two studies have been designed to provide in this by studying trajectories of neurodevelopment and behavioral outcomes in the first few years of life, and by looking into predictors of positive and negative

outcomes; the TRIXY Early Childhood study, Leiden University, the Netherlands, and the eXtraordinarY babies study, Denver, USA (Tartaglia et al., 2020).

To conclude, our results show that already at a young age, language is a vulnerable domain in children with SCT. Both receptive and expressive language can be affected and should be monitored closely. More longitudinal studies are needed that investigate the impact of early language interventions on later language outcomes. Finally, interventions should be implemented as soon as needed, to prevent more severe problems in later life.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.) [Text Revision]. American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596

Bayley, N. (2006). *Bayley scales of infant and toddler development - third edition*. Harcourt Assessment

Bishop, D. V. M., Brookman-Byrne, A., Gratton, N., Gray, E., Holt, G., Morgan, L., . . . Thompson, P. A. (2018). Language phenotypes in children with sex chromosome trisomies. *Wellcome Open Research*, *3*, 143. https://doi.org/10.12688/wellcomeopenres.14904.2

Boada, R., Janusz, J., Hutaff-Lee, C., & Tartaglia, N. (2009). The cognitive phenotype in Klinefelter syndrome: A review of the literature including genetic and hormonal factors. *Developmental Disabilities Research Reviews*, *15*(4), 284-294. https://doi.org/10.1002/ddrr.83

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevelance of Klinefelter syndrome: A national registry study. *The Journal of Clinical Endocrinology & Metabolism*, *88*(2), 622-626. https://doi.org/10.1210/jc.2002-021491

Bryant, D. M., Hoeft, F., Lai, S., Lackey, J., Roeltgen, D., Ross, J., & Reiss, A. L. (2012). Sex chromosomes and the brain: A study of neuroanatomy in XYY syndrome. *Developmental Medicine and Child Neurology*, *54*(12), 1149-1156. https://doi.org/10.1111/j.1469-8749.2012.04418.x

Capone, N. C., & McGregor, K. K. (2004). Gesture development: A review for clinical and research practices. *Journal of Speech, Language, and Hearing Research*, *47*, 173-186. https://doi.org/10.1044/1092-4388(2004/015)

Dunn, L. M., & Dunn, L. M. (1997). *Peabody Picture Vocabulary Test - Third Edition Manual*. American Guidance Service

Dunn, L. M., & Dunn, L. M. (2005). *Peabody Picture Vocabulary Test-III-NL. Handleiding* (L. Schlichting, Trans.). Harcourt Test Publishers.

Eriksson, M., Marschik, P. B., Tulviste, T., Almgren, M., Pérez Pereira, M., Wehberg, S., . . . Gallego, C. (2012). Differences between girls and boys in emerging language skills: Evidence from 10 language communities. *British Journal of Developmental Psychology*, *30*, 326-343. https://doi.org/10.1111/j.2044-835X.2011.02042.x

Fenson, L., Marchman, V. A., Thal, D. J., Dale, P. S., Reznick, J. S., & Bates, E. (2007). *MacArthur-Bates Communicative Development Inventories: User's guide and technical manual (2nd ed.)*. Baltimore, MD: Brookes

Geschwind, D. H., Boone, K., Miller, B. L., & Swerdloff, R. S. (2000). Neurobehavioral phenotype of Klinefelter syndrome. *Mental Retardation and Developmental Disabilities Research Reviews*, *6*, 107-116. https://doi.org/10.1002/1098-2779(2000)6:2<107::AID-MRDD4>3.0.CO;2-2

Giedd, J. N., Clasen, L. S., Wallace, G. L., Lenroot, R. K., Lerch, J. P., Wells, E. M., . . . Samango-Sprouse, C. A. (2007). XXY (Klinefelter syndrome): A pediatric quantitative brain magnetic resonance imaging case-control study. *Pediatrics*, *119*(1), e232-240. https://doi.org/10.1542/peds.2005-2969

Groth, K. A., Skakkebaek, A., Høst, C., Gravholt, C. H., & Bojesen, A. (2013). Klinefelter syndrome - a clinical update. *The Journal of Clinical Endocrinology & Metabolism*, *98*(1), 20-30. https://doi.org/10.1210/jc.2012-2382

Hollingshead, A. B. (1975). *Four factor index of social status* [Unpublished Manuscript]. Yale University, New Haven, CT.

Hong, D. S., & Reiss, A. L. (2014). Cognitive and neurological aspects of sex chromosome aneuploidies. *The Lancet Neurology*, *13*(3), 306-318. https://doi.org/10.1016/s1474-4422(13)70302-8

Korkman, M., Kirk, U., & Kemp, S. L. (2007a). *NEPSY II. Administrative manual*. Psychological Corporation.

Korkman, M., Kirk, U., & Kemp, S. L. (2007b). *NEPSY II. Clinical and Interpretative manual*. Psychological Corporation.

Lee, N. R., Wallace, G. L., Adeyemi, E. I., Lopez, K. C., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2012). Dosage effects of X and Y chromosomes on language and social functioning in children with supernumerary sex chromosome aneuploidies: Implications for idiopathic language impairment and autism spectrum disorders. *Journal of Child Psychology and Psychiatry*, *53*(10), 1072-1081. https://doi.org/10.1111/j.1469-7610.2012.02573.x

Leggett, V., Jacobs, P., Nation, K., Scerif, G., & Bishop, D. V. (2010). Neurocognitive outcomes of individuals with a sex chromosome trisomy: XXX, XYY, or XXY: A systematic review. *Developmental Medicine & Child Neurology*, *52*(2), 119-129. https://doi.org/10.1111/j.1469-8749.2009.03545.x

Lenroot, R. K., Blumenthal, J. D., Wallace, G. L., Clasen, L. S., Lee, N. R., & Giedd, J. N. (2014). A case-control study of brain structure and behavioral characteristics in 47,XXX syndrome. *Genes Brain and Behavior*, *13*(8), 841-849. https://doi.org/10.1111/gbb.12180

Levey, S. (2019). *An introduction to language development, Second edition*. Plural Publishing, Inc., San Diego

Lezak, M. D., Howieson, D., Loring, D., & Hannay, H. (2004). *Neuropsychological assessment* (4th ed.). Oxford University Press.

Mandoki, M. W., Sumner, G. S., Hoffman, R. P., & Riconda, D. L. (1991). A review of Klinefelter's syndrome in children and adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, *30*(2), 167-172. https://doi.org/10.1097/00004583-199103000-00001

Morris, J. K., Alberman, E., Scott, C., & Jacobs, P. (2008). Is the prevalence of Klinefelter syndrome increasing? *European Journal of Human Genetics*, *16*, 163-170. https://doi.org/10.1038/sj.ejhg.5201956

Otter, M., Schrander-Stumpel, C. T., & Curfs, L. M. (2010). Triple X syndrome: A review of the literature. *European Journal of Human Genetics*, *18*(3), 265-271. https://doi.org/10.1038/ejhg.2009.109

Owens Jr., R. E. (2011). *Language development: An introduction* (8th ed., International ed. ed.). Pearson Education.

Raznahan, A., Lee, N. R., Greenstein, D., Wallace, G. L., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2016). Globally divergent but locally convergent X- and Y-chromosome influences on cortical development. *Cerebral Cortex*, *26*, 70-79. https://doi.org/10.1093/cercor/bhu174

Re, L., & Birkhoff, J. M. (2015). The 47,XYY syndrome, 50 years of certainties and doubts: A systematic review. *Aggression and Violent Behavior*, *22*, 9-17. https://doi.org/10.1016/j.avb.2015.02.003

Robinson, A., Bender, B., Borelli, J., Puck, M., & Salbenblatt, J. (1983). Sex chromosomal anomalies: Prospective studies in children. *Behavior Genetics*, *13*(4), 321-329. https://doi.org/10.1007/BF01065770

Ross, J. L., Kushner, H., Kowal, K., Bardsley, M., Davis, S., Reiss, A. L., . . . Roeltgen, D. (2017). Androgen treatment effects on motor function, cognition, and behavior in boys with Klinefelter syndrome. *The Journal of Pediatrics*, *185*, 193-199.e194. https://doi.org/10.1016/j.jpeds.2017.02.036

Ross, J. L., Roeltgen, D. P., Stefanatos, G., Benecke, R., Zeger, M. P. D., Kushner, H., . . . Zinn, A. R. (2008). Cognitive and motor development during childhood in boys with Klinefelter syndrome. *American Journal of Medical Genetics Part A*, *146A*(6), 708-719. https://doi.org/10.1002/ajmg.a.32232

Ross, J. L., Zeger, M. P., Kushner, H., Zinn, A. R., & Roeltgen, D. P. (2009). An extra X or Y chromosome: contrasting the cognitive and motor phenotypes in childhood in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Developmental Disabilities Research Reviews*, *15*(4), 309-317. https://doi.org/10.1002/ddrr.85

Rourke, B. P., Bakker, D. J., Fisk, J. L., & Strang, J. D. (1983). *Child neuropsychology: Introduction to theory, research, and clinical practice*. Guilford Press.

Simms, M. D. (2007). Language disorders in children: Classification and clinical syndromes. *Pediatric Clinics of North America*, *54*, 437-467. https://doi.org/10.1016/j.pcl.2007.02.014

Skakkebaek, A., Gravholt, C. H., Chang, S., Moore, P. J., & Wallentin, M. (2020). Psychological functioning, brain morphology, and functional neuroimaging in Klinefelter syndrome. *American Journal of Medical Genetics part C*, *184*(2), 506-517. https://doi.org/10.1002/ajmg.c.31806

St John, M., Ponchard, C., van Reyk, O., Mei, C., Pigdon, L., Amor, D. J., & Morgan, A. T. (2019). Speech and language in children with Klinefelter syndrome. *Journal of Communication Disorders*, *78*, 84-96. https://doi.org/10.1016/j.jcomdis.2019.02.003

Tartaglia, N., Howell, S., Davis, S., Kowal, K., Tanda, T., Brown, M., . . . Ross, J. (2020). Early neurodevelopmental and medical profile in children with sex chromosome trisomies: Background for the prospective eXtraordinarY babies study to identify early risk factors and targets for intervention. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *184*(2), 428-443. https://doi.org/10.1002/ajmg.c.31807

Tartaglia, N. R., Howell, S., Sutherland, A., Wilson, R., & Wilson, L. (2010). A review of trisomy X (47,XXX). *Orphanet Journal of Rare Diseases*, *5*, 8. https://doi.org/10.1186/1750-1172-5-8

Urbanus, E., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2020). The behavioral profile of children aged 1-5 years with sex chromosome trisomy (47,XXX, 47,XXY, 47,XYY). *American Journal of Medical Genetics part C*, *184C*, 444-455. https://doi.org/10.1002/ajmg.c.31788

Urbanus, E., Van Rijn, S., & Swaab, H. (2019). A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention. *Clinical Genetics*, *97*(1), 156-167. https://doi.org/10.1111/cge.13586

Van Rijn, S. (2019). A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47,XXX, 47,XYY). *Current Opinion in Psychiatry*, *32*(2), 79-84. https://doi.org/10.1097/yco.0000000000000471

Van Rijn, S., Aleman, A., Swaab, H., Vink, M., Sommer, I., & Kahn, R. S. (2008). Effects of an extra X chromosome on language lateralization: An fMRI study with Klinefelter men (47,XXY). *Schizophrenia Research*, *101*(1-3), 17-25. https://doi.org/10.1016/j.schres.2008.02.001

Verri, A., Cremante, A., Clerici, F., Destefani, V., & Radicioni, A. (2010). Klinefelter's syndrome and psychoneurologic function. *Molecular Human Reproduction*, *16*(6), 425-433. https://doi.org/10.1093/molehr/gaq018

Visootsak, J., & Graham Jr., J. M. (2006). Klinefelter syndrome and other sex chromosomal aneuploidies. *Orphanet Journal of Rare Diseases*, *1*, 42. https://doi.org/10.1186/1750-1172-1-42

Wechsler, D. (2002). *WPPSI-III: Technical and interpretative manual* The Psychological Corporation

Wechsler, D., & Naglieri, J. A. (2006). *Wechsler Nonverbal Scale of Ability*. Harcourt Assessment

Wiig, E. H., Secord, W. A., & Semel, E. (2004). *Clinical Evaluation of Language Fundamentals - Preschool (2nd ed.)*. Harcourt Assessment

Wiig, E. H., Secord, W. A., & Semel, E. (2012). *Clinical Evaluation of Language Fundamentals Preschool (CELF-2NL) - Nederlandse Versie* (J. de Jong, Trans.). Pearson Assessment and Information.

Wilson, A. C., & Bishop, D. V. M. (2018). Sex chromosome trisomies are not associated with atypical lateralization for language. *Developmental Medicine and Child Neurology*, *60*(11), 1132-1139. https://doi.org/10.1111/dmcn.13929

Zampini, L., Burla, T., Silibello, G., Capelli, E., Dall'Ara, F., Rigamonti, C., . . . Vizziello, P. G. (2020). Preverbal skills in 8-month-old children with sex chromosome trisomies. *First Language*, *41*(2), 200-217. https://doi.org/10.1177/0142723720962944

Zampini, L., Burla, T., Silibello, G., Dall'Ara, F., Rigamonti, C., Lalatta, F., & Vizziello, P. (2017). Early communicative skills of children with Klinefelter syndrome. *Clinical Linguistics & Phonetics*, *32*(7), 577-586. https://doi.org/10.1080/02699206.2017.1384853

Zampini, L., Draghi, L., Silibello, G., Dall'Ara, F., Rigamonti, C., Suttora, C., . . . Vizziello, P. (2018). Vocal and gestural productions of 24-month-old children with sex chromosome trisomies. *International Journal of Language & Communication Disorders*, *53*(1), 171-181. https://doi.org/10.1111/1460-6984.12334

Zink, I., & Lejaegere, M. (2014). *N-CDIs: Lijsten voor Communicatieve Ontwikkeling, aanpassing en Hervorming van de MacArthur CDIs van Fenson et al.* Vlaamse Vereniging voor Logopedisten.

Supplementary Materials

Correlation Matrices Background Information and Outcome Measures per Age Group

Table A1. Correlation table 1-year-olds: SES, parental age, and language outcomes

	1. SES	2. Parental Age	3. Receptive Vocabulary	4. Expressive Vocabulary	5. Receptive Semantics	6. Expressive Semantics
1. SES	-	.23	.08	.22	.32	.27
2. Parental Age	.18	-	.08	-.13	.29	.16
3. Receptive Vocabulary	.03	.13	-	.68**	.31	.43*
4. Expressive Vocabulary	.12	-.03	.06	-	.29	.43*
5. Receptive Semantics	.11	-.01	.65**	-.26	-	.85**
6. Expressive Semantics	-.09	.14	.46*	-.16	.85**	-

Note: Numbers under diagonal (uncolored) represent correlations in the SCT 1-year old group.
Numbers above the diagonal (marked in grey) represent correlations in the Control 1-year old group.
* $p < .05$, ** $p < .01$ (2-tailed)

Table A2. Correlation table 3-4-year-olds: SES, parental age, nonverbal IQ, and language outcomes

	1. SES	2. Parental Age	3. Nonverbal IQ	4. Receptive Semantics	5. Expressive Semantics	6. Syntax	7. Phonological Processing
1. SES	-	.38**	.24	.29	.20	.19	.25
2. Parental Age	-.17	-	.19	.26	.06	.19	.15
3. Nonverbal IQ	.39*	-.26	-	.32*	.18	.30*	-.01
4. Receptive Semantics	.27	-.18	.41*	-	.68**	.71**	.54**
5. Expressive Semantics	.14	-.25	.25	.74**	-	.65**	.49**
6. Syntax	.31	.04	.53**	.68**	.40*	-	.44**
7. Phonological Processing	.31	-.01	.28	.59**	.35*	.60**	-

Note: Numbers under diagonal (uncolored) represent correlations in the SCT 3-4-year old group.
Numbers above the diagonal (marked in grey) represent correlations in the Control 3-4-year old group.
* $p < .05$, ** $p < .01$ (2-tailed)

Table A3. Correlation table 5-6-year-olds: SES, parental age, nonverbal IQ, and language outcomes

	1. SES	2. Parental Age	3. Nonverbal IQ	4. Receptive Semantics	5. Expressive Semantics	6. Syntax	7. Phonological Processing
1. SES	-	.26	.34	.21	.14	-.07	.03
2. Parental Age	.13	-	.15	.16	.25	.39	.05
3. Nonverbal IQ	.36	-.14	-	.08	.07	.35	.24
4. Receptive Semantics	.34	.05	.51*	-	.62**	.17	.23
5. Expressive Semantics	.04	.18	.43*	.77**	-	.51**	.36
6. Syntax	.41*	.06	.54**	.65**	.65**	-	.46*
7. Phonological Processing	.12	-.07	.59**	.74**	.67**	.57**	-

Note: Numbers under diagonal (uncolored) represent correlations in the SCT 5-6-year old group.
Numbers above the diagonal (marked in grey) represent correlations in the Control 5-6-year old group.
* $p < .05$, ** $p < .01$ (2-tailed)

Chapter 5

Structural and pragmatic language in young children with sex chromosome trisomy (XXX, XXY, XYY): Predictive value for neurobehavioral problems one year later

Accepted as:

Urbanus, E., Swaab, H., Tartaglia, N., Stumpel, C., & Van Rijn, S. Structural and pragmatic language in young children with sex chromosome trisomy (XXX, XXY, XYY): Predictive value for neurobehavioral problems one year later. *The Clinical Neuropsychologist.* https://doi.org/10.1080/13854046.2022.2067078

Abstract

Objective. To investigate pragmatic language abilities in young children with an increased risk for adverse neurobehavioral and neurocognitive outcomes due to an extra X or Y chromosome (sex chromosome trisomy; SCT) and to investigate to what degree early structural and pragmatic language abilities are predictive of neurobehavioral problems one year later.

Method. In total, 72 children with SCT and 71 controls aged 3-7 years were included. Language assessments included parent-reported pragmatic language skills and direct assessment of structural language abilities. Parent-reported behavioral outcomes were measured one year after the initial language assessment.

Results. Children with SCT demonstrated weaker pragmatic language skills compared to controls. These differences were not driven by karyotype, time of diagnosis, or ascertainment bias and irrespective of the presence of structural language impairment. Odds of having pragmatic difficulties was 23 times higher in the SCT group, with 25% of the children not meeting age-expectations. In addition, language, in particular pragmatic language, was an important predictor for later affective, oppositional defiant, pervasive developmental, attention deficit, and social-emotional problems in young children with SCT.

Conclusions. This study is one of the first studies that directly illustrates the relationship between language and behavioral outcomes in children with SCT. Our results stress the importance to closely monitor pragmatic language in addition of structural language in clinical care of children with SCT, as pragmatic language abilities could serve as an early marker for children at risk for developing behavioral problems.

Introduction

Approximately 1 in 650 to 1 in 1000 children is born with an extra X or Y chromosome, or sex chromosome trisomy (SCT; Bojesen et al., 2003; Groth et al., 2013; Morris et al., 2008). This leads to a 47,XXY or 47,XYY pattern in males or a 47,XXX pattern in females. SCT is a relatively common genetic variation, associated with an increased risk for neurocognitive difficulties (for a review see Urbanus et al., 2019), neurodevelopmental disorders (for a review see Van Rijn, 2019) and for social-emotional and behavioral problems (Urbanus et al., 2020). As children with SCT can be diagnosed prenatally, this gives a unique opportunity to prospectively follow a group of children from an early age who biologically have a heightened risk to develop neurodevelopmental difficulties, and to investigate mechanisms of developmental vulnerability. It is likely that neurodevelopmental difficulties are anchored in early brain maturation; on both the X and the Y chromosome, genes are located that play an important role in neural development and cognitive functioning (Lenroot et al., 2014; Raznahan et al., 2016). Global intellectual functioning is variable in children with SCT, ranging from impaired to above average with mean functioning in the average to low-average range (for a review see Urbanus et al., 2019). Some studies found relative strengths in non-verbal reasoning and spatial intellectual functioning in contrast to performance on verbal intellectual tests (e.g., Cordeiro et al., 2012; Netley, 1986; Ross et al., 2008; Rovet et al., 1995; Rovet et al., 1996). In addition, neurocognitive difficulties have been reported in children with SCT regardless of level of intellectual functioning.

Neurocognitive functions could serve as early markers for behavioral problems in later life. Knowledge about early neurocognitive functions that underlie behavioral outcomes is important, as these functions could serve as important targets for early treatment and intervention. Among these neurocognitive difficulties are disturbances in language development, with studies reporting language difficulties in 70-80% of included SCT individuals (Boada et al., 2009). Recent studies including very young children with SCT indicate that these language difficulties can already be identified before children are one-year old (Urbanus et al., 2021; Zampini et al., 2020). Language problems are considered one of the most prominent neurocognitive vulnerabilities associated with SCT. Recent studies have shown difficulties in areas of early non-verbal communication (Zampini et al., 2017), early vocabulary (Zampini et al., 2017; Zampini et al., 2018), and semantic skills (Ross et al., 2008; Ross et al., 2009; St John et al., 2019). However, the primary focus within these studies has been on structural aspects of language, which encompasses all aspects of language related to form (i.e., phonology, morphology, syntax) and content (i.e., semantics), whereas the use of language in a social context or *pragmatic languag*e is also important for social interaction and communicating with others.

Pragmatic language consists of a variety of skills; these include understanding and use of communicative intentions, presupposition, and discourse management. Pragmatic language encompasses paralinguistic and nonverbal aspects of language (Parsons et al., 2017). For example, in conversation it is important to take the other's needs into account and to adapt to these needs if necessary (Asada et al., 2010). Within the SCT population, pragmatic language has been largely understudied. One study of boys with XXY aged 1-18 years reported deficits in pragmatic language that were more pronounced in older children (St John et al., 2019). Another study reported lower pragmatic language skills, including inappropriate initiation of conversation, difficulties with understanding and using scripted language, and difficulties with

nonverbal communication, in children and adolescents (aged 4-22 years) with an extra X or Y chromosome compared to typically developing peers (Lee et al., 2012). Two studies with children and adolescents aged 5-16 also reported increased rates of pragmatic language difficulties in all three karyotypes, including inappropriate initiation of conversation, difficulties with using conversational contexts, and difficulties with nonverbal communication. The authors reported more pronounced difficulties in subgroups of children with a postnatal diagnosis or children with behavioral or neurodevelopmental problems (Bishop et al., 2018; Bishop et al., 2011). In addition, there is some evidence that 'higher order language levels' are affected in children with SCT, such as understanding of ambiguous sentences, figurative speech, and understanding meaning in context (Melogno et al., 2019; Ross et al., 2008; Ross et al., 2009).

Both structural and pragmatic aspects of language are part of the larger concept of communication. Adequate communication depends not only on structural language abilities, but also on one's ability to use language in a social context. Studies have shown a relationship between structural and pragmatic language and behavioral outcomes in a diverse range of populations. Children with developmental language delays show more behavioral problems (Gallagher, 1999) and early language difficulties are commonly reported in children with (neuro)developmental disorders such as autism spectrum disorders (ASD; Miranda et al., 2020; Volden et al., 2009), attention-deficit hyperactivity disorder (ADHD; Staikova et al., 2013), oppositional defiant disorder (Gremillion & Martel, 2014) and conduct disorder (Gilmour et al., 2004).

Studies have pointed at an increased risk for psychopathology in individuals with SCT, including risks for ASD and ADHD (see for example, (Ross et al., 2012; Samango-Sprouse et al., 2018; Tartaglia et al., 2010; Urbanus et al., 2020; Van Rijn, 2019; van Rijn, Stockmann, Borghgraef, et al., 2014)). Although it has been suggested that (structural) language difficulties could be linked to social difficulties in later life (Visootsak & Graham, 2009), studies that investigate the relationship between language and behavioral outcomes are lacking. In addition, to fully understand the relationship between language and risk for behavioral and social-emotional problems, it is important to take into account not only structural language, but pragmatic language as well. Lastly, by studying this relationship in young children, building blocks of later behavioral outcomes can be identified, which is important to identify targets for early interventions.

The present study focuses on pragmatic language abilities in young children with SCT (aged 3-7 years) and investigates the role of structural and pragmatic language in predicting behavioral outcomes one year later. The aims of this paper are two-fold. First, to determine if the presence of an extra X or Y chromosome not only affects structural language development, but also affects pragmatic skills in young children. In other words: Do children with SCT have communication deficits beyond structural language? Several questions will be answered to pinpoint which children are vulnerable for adverse pragmatic language outcomes: (1) Do children with SCT have similar pragmatic abilities compared to controls? Factors that could be relevant for interpretation of the results (e.g., specific SCT karyotype, time of diagnosis, ascertainment bias) were explored. (2) Is the proportion of children with age-appropriate pragmatic skills similar in both groups? (3) Within the SCT group, do only children with structural language problems experience problems with pragmatic language or are pragmatic language difficulties a more common deficit within this group? (4) Is the developmental

pathway of pragmatic language skills comparable in children with and without SCT? The second aim of this paper is to determine if language abilities predict neurobehavioral outcomes in later development; more specifically, if pragmatic language abilities can predict these outcomes above and beyond the predictive value of structural language abilities.

As children with SCT have a biological risk to develop language difficulties and have an increased risk for unfavorable behavioral outcomes, it is important to investigate possible underlying mechanisms of these behavioral outcomes, for example early language and communication abilities. Focusing on pragmatic language, thus considering communication in a broader perspective than structural language alone, may yield important insights in this, and could help identify early markers for children with vulnerable behavioral development.

Materials and Methods

Participants

The present study is part of a larger ongoing project (TRIXY Early Childhood Study) at Leiden University, which included children with SCT and nonclinical controls aged 1-7 years. The TRIXY Early Childhood Study is a longitudinal study that aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. For the present study, both children with SCT and children in the control group aged 3-7 years during the initial visit were included.

Clinical genetic departments, pediatricians, and national advocacy or support groups in the Netherlands, Colorado USA, and Belgium participated in the recruitment of children with SCT. Assessment took place in the Netherlands (Trisomy of the X and Y – TRIXY – Expert Center) and the USA (Children's Hospital Colorado eXtraordinarY Kids Clinic in Developmental Pediatrics at University of Colorado). The control group was recruited in the western part of the Netherlands. With the help of government institutions, the civil registry was accessed, and information brochures were distributed among families with children of eligible age. In addition, public sites such as daycare centers and public schools were asked to distribute information brochures as well. If parents were interested in the study, they were able to contact the researchers to discuss enrollment.

In both participant groups, the child as well as the (primary) parent/caregiver had to speak Dutch or English. Children were excluded when there was a history of traumatic brain injury, severely impaired hearing or sight, neurological illness, or colorblindness. Specific for the SCT group, the trisomy had to be present in at least 80% of the cells (confirmed by standard karyotyping). Within the control group genetic screening was not performed due to ethical reasons. However, based on the prevalence of SCT, the risk of a SCT karyotype in the control group was considered minimal and acceptable.

In the present study, 72 children with SCT (M_{age} = 4.80, SD = 1.29) and 71 children without SCT (M_{age} = 4.51, SD = .99) were included. There were no significant age or age-distribution differences between the children with SCT and controls (p = .138), nor were there differences in average age between children with XXX, XXY, or XYY (p = .605). Global intellectual functioning (GIF) was assessed with the Wechsler Preschool and Primary Scale of Intelligence (third edition; Wechsler, 2002), or the Wechsler Nonverbal Scale of Ability (Wechsler & Naglieri, 2006). There was a significant difference in average GIF between the SCT and control group (p < .001, Cohen's d = .81), but no significant difference between children with XXX, XXY, or XYY (p = .304). Highest level of parental education was used as

an indication of socio-economic status (SES), if a child had two caregivers, SES was calculated as an average for both caregivers. There was a significant difference in SES between the SCT and control group (p = .021, Cohen's d = .39); parents of children with SCT had higher levels of education. There were no significant differences in SES between children with XXX, XXY, or XYY (p = .525). Descriptive statistics for age, GIF, and SES can be found in Table 1.

Within the SCT group, both time of diagnosis and ascertainment bias were assessed. Regarding time of diagnosis, 40 children received the diagnosis prenatally (i.e., because of prenatal screening or advanced maternal age). Of the children that received a postnatal diagnosis (N = 32), reasons for genetic screening included developmental delay (N = 14), physical and/or growth problems (N = 9), or medical concerns (N = 9). Regarding ascertainment bias, children were divided into three subgroups: A) 'Active prospective follow-up' (43.1%), including families that were actively followed after a prenatal diagnosis; B) 'Information seeking parents' (29.2%), including families who enrolled into the study to learn more about their child's condition, but without having specific concerns about their child's development, and C) 'Clinically referred cases' (27.8%), including families who enrolled into the study after receiving professional help because of specific concerns about the development of their child. The distribution of prenatal and postnatal diagnoses was similar across the three karyotypes (p = .998). There were no differences in the distribution of ascertainment bias across the three SCT karyotypes (p = .232). Descriptives of time of diagnosis and ascertainment bias can be found in Table 1.

Behavioral outcomes one year after initial assessment were studied. Data was available for 48 children with SCT (23 XXY, 16 XXX, 9 XYY) and 58 children in the control group. The high number of dropouts was mostly due to the worldwide COVID-19 pandemic, where assessments had to be canceled or postponed (N_{SCT} = 16; $N_{control}$ = 5), other reasons for dropout were developmental concerns (N_{SCT} = 2; $N_{control}$ = 1), family circumstances (N_{SCT} = 1), or the child being too old for the specific assessment battery (N_{SCT} = 2; $N_{control}$ = 1). For the remaining participants, reason for dropout was unknown (N_{SCT} = 3; $N_{control}$ = 6). On average, the behavioral assessments took place 52 weeks after the initial assessment (range 50-61 weeks). Ages during the follow-up assessment ranged from 4.08-8.03 years (M_{age} = 5.61, SD = 1.07). Baseline scores for neurocognitive and behavioral outcomes were compared between SCT children who were included in the follow-up assessment and children with missing follow-up data. Multivariate analyses of variance indicated no significant multivariate difference for cognitive outcomes (i.e., GIF, structural language, pragmatic language), Wilk's Lambda = .98, F(3,61) = .42, p = .738, partial η^2 = .02, or behavioral outcomes that were available for the entire age range, Wilk's Lambda = .93, F(5,64) = 1.00, p = .428, partial η^2 = .07. Participant demographics, neurocognitive outcomes and behavioral outcomes of the initial assessment are reported in Table 2 for the entire SCT group and the SCT group with follow-up data.

Table 1. Descriptives: Children with sex chromosome trisomy versus controls

	SCT	XXX	XXY	XYY	Control	XX	XY	*p*[d]	SCT comparisons[e]
Total *N*	72	27	29	16	71	40	31		
Age – Mean (SD)	4.80 (1.29)	4.89 (1.19)	4.61 (1.35)	4.98 (1.40)	4.51 (.99)	4.53 (1.08)	4.49 (.90)	.138	n.s.
Global intellectual functioning[a]	95.19 (19.37)	92.85 (16.88)	99.59 (20.11)	91.07 (21.89)	108.31 (13.80)	106.50 (12.81)	110.65 (14.86)	<.001	n.s.
Range	55-138	60-122	55-138	59-125	72-140	76-137	72-40		
Socio-economic status[b]	5.90 (.96)	5.94 (.93)	5.98 (.95)	5.66 (1.03)	5.42 (1.45)	5.21 (1.37)	5.68 (1.52)	.021	n.s.
Time of Diagnosis (prenatal/postnatal)	40/32	15/12	16/13	9/7					n.s.
Ascertainment bias (A/B/C)[c]	31/21/20	7/10/10	15/8/6	9/3/4					n.s.

Note: Scores represent Means (SD)
Abbreviations: n.s. = not significant; SCT = Sex Chromosome Trisomy
[a] Measured with the WPPSI-III or the Wechsler Nonverbal Scale of Ability; Data for 5 children with SCT was incomplete (1 XXX, 2 XXY, 2 XYY)
[b] Classified according to the criteria of Hollingshead: 0) No formal education; 1) Less than 7th grade; 2) Junior high school; 3) Partial high school; 4) High school graduate; 5) Partial college or specialized training; 6) Standard college/university graduation; 7) Graduate/professional training
[c] A = Active prospective follow-up, B = Information seeking parents, C = Clinically referred
[d] *p*-value SCT versus Control comparison
[e] SCT comparisons: XXX versus XXY versus XYY

Table 2. Baseline descriptives for children with sex chromosome trisomy: Total baseline group versus group included in predictive analyses (one-year follow-up)

				Baseline			Follow-up	
	Measure	**Instrument**	**N**	**M (SD)**		**N**	**M (SD)**	
Demographics	Age (years)		72	4.80 (1.29)		48	4.67 (1.19)	
	Socio-economic status	Hollingshead criteria[a]	72	5.90 (.96)		48	5.95 (1.01)	
	Research site[b]	Dutch speaking	33			21		
		English speaking	39			27		
				Baseline			**Follow-up**	
	Measure	**Instrument**	**N**	**Raw score M (SD)**	**Standardized score M (SD)**	**N**	**Raw score M (SD)**	**Standardized score M (SD)**
Cognitive outcomes	Global intellectual functioning	Standardized total IQ score measured with WPPSI-III or Wechsler Nonverbal Scale of Ability	67	N/A	95.19 (19.37)	44	N/A	96.75 (18.27)
	Structural language	Combined z-score of expressive semantic skills (CELF-P), receptive semantic skills (PPVT), and syntax (CELF-P)	69	N/A	-.32 (.85)	46	N/A	-.23 (.85)
	Pragmatic language	Total raw score measured with CELF-P pragmatics profile	72	76.17 (12.58)	N/A	48	76.44 (12.95)	N/A
Behavioral outcomes	Anxiety	Corrected raw score[c] and standardized T score measured with CBCL-DSM scales	70	22.29 (20.56)	57.36 (9.71)	48	18.99 (18.11)	56.19 (8.94)
	Affective		70	17.13 (12.57)	58.67 (8.07)	48	16.43 (12.27)	58.15 (7.70)
	Oppositional defiant		70	37.71 (25.75)	57.07 (8.32)	48	37.74 (25.46)	57.04 (8.12)
	Attention deficit		70	39.01 (22.88)	54.47 (6.11)	48	38.22 (23.64)	54.15 (6.08)
	Pervasive developmental		55	25.80 (16.19)	63.29 (9.85)	40	26.63 (17.36)	63.73 (10.35)
	Social-emotional	Corrected raw score[c] measured with ASQ-SE-2	71	13.22 (11.55)	N/A	48	12.61 (11.21)	N/A

Abbreviations: N/A = not applicable

[a] Hollingshead criteria: 0) No formal education; 1) Less than 7th grade; 2) Junior high school; 3) Partial high school; 4) High school graduate; 5) Partial college or specialized training; 6) Standard college/university graduation; 7) Graduate/professional training

[b] The testing set-up and research protocols were identical for all sites to permit standardization of the testing set-up. The same instruments were used on all sites; tests and questionnaires were administered in either Dutch or English.

[c] Raw scores were corrected for the maximum possible score and multiplied by 100 to correct for differences in the number of items between age specific forms

Procedure

This study was approved by the Ethical committee of Leiden University Medical Center, the Netherlands, and the Colorado Multiple Institutional Review Board (COMIRB) in Colorado, USA. Written informed consent according to the declaration of Helsinki was obtained after providing a description of the study to the parent(s) of the child.

The primary caregiving parent (92% biological mother) was asked to complete several questionnaires, including questionnaires regarding social-emotional, behavioral, and language outcomes. The child was assessed either in a quiet room at the university or at home. Assessment took place at various sites (Colorado USA, the Netherlands, Belgium). The testing set-up and research protocols were identical on all sites to permit standardization of the testing set-up. Researchers from Leiden University were responsible for project and data management (i.e., training and supervision of researchers, processing and scoring of data).

Due to the inclusion of participants from multiple sites, the tasks and questionnaires were administered in either Dutch or English. Tasks and questionnaires in both languages are formally validated and have sufficient psychometric properties. When applicable language-specific norms based on population samples were used.

Instruments

Structural Language

Receptive language skills were assessed with the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997, 2005). Expressive language skills with the Expressive Vocabulary subtest of the Clinical Evaluation of Language Fundamentals Preschool edition (CELF-P EW) and syntax with the Sentence Structure subtest of the CELF-P (CELF-P SS; Wiig et al., 2004, 2012),

The PPVT assesses the child's ability to comprehend spoken words. For each item, four black and white pictures were shown to the child, and the child was instructed to identify the word that was orally presented by the researcher. The CELF-P EV test assesses the child's ability to label people, objects, and actions by looking at colored images. The CELF-P SS test assesses the child's ability to interpret sentences of increasing length and structural complexity by identifying a picture out of four option that illustrates the orally presented sentence.

Pragmatic Language

The primary caregiving parent of the child completed the pragmatics profile of the CELF-P (Wiig et al., 2004, 2012). The CELF-P pragmatics profile is a checklist including 26 statements that the parent rates on a 4-point scale (never, sometimes, often, always). The pragmatics profile assesses three subdomains: 1) The child's non-verbal communication abilities (7 statements; e.g., the child appropriately responds to a familiar person's angry, happy, or sad tone of voice), 2) the child's ability to request, give, and respond to information (12 statements; e.g., the child appropriately asks questions if he or she is confused), and 3) the child's conversational routines and skills (7 statements; e.g., the child appropriately introduces new conversation topics). Answers for the statements on the three subdomains were added to total sub-scores and answers on all statements were summed to a total (raw) score. Higher scores indicate better pragmatic abilities.

Behavioral Outcomes

The primary caregiving parent of the child completed two questionnaires to assess behavioral outcomes: The Ages-and-Stages Social-Emotional Questionnaire (ASQ-SE-2; Squires et al., 2015) and the Child Behavior Checklist (CBCL; Achenbach & Ruffle, 2000). For both questionnaires, the primary caregiving parent completed the age-appropriate version.

The ASQ-SE-2 assesses social- and emotional development on seven behavioral constructs. The used form depends on the age of the child, with number of questions ranging from 19-33. Items were answered on a 3-point scale (rarely or never, sometimes, most of the time) and for each item parents indicated if the specific behavior was a concern. Answers on the items and the number of concerns indicated add up to a total raw score, with higher scores indicating an increased risk for social-emotional deficits or delays.

The CBCL is a standardized measure of behavioral problems. Answers on the items yield several outcomes, including the DSM-oriented scales. Depending on the used form (i.e., 1.5-5 or 6-18 years), the DSM-oriented scales consist of five or six profiles. In this study, the following profiles were assessed (with number of items on the 1.5-5- and 6-18-year version respectively): 1) Affective problems (as an indication for mood disorders, 10/13 items), 2) Anxiety problems (10/6 items), 3) Pervasive developmental problems (as indication of disorders on the autism spectrum, included in 1.5-5 year old version only), 4) Attention deficit/hyperactivity problems (6/7 items), and 5) Oppositional defiant problems (6/5 items). Items were answered on a 3-point scale (not true, somewhat or sometimes true, very true or often true), with higher scores indicating more behavioral problems.

As the number of total items differs between the ASQ-SE-2 versions and between the CBCL 1.5-5 and 6-18 years, raw scores were corrected for the maximum possible score and multiplied by 100. Raw scores were preferred due to greater variability in scores and as raw scores are more appropriate for parametric statistical analyses. By correcting these scores, we were able to include children of all ages in the analyses (with the exception of the DSM pervasive developmental problems scale), with higher scores denoting more problems. Due to the small sample of children with scores on the CBCL somatic problems and conduct problems ($N < 20$), these scales were discarded.

Statistical Analyses

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) Version 25. Level of significance was set at $p \leq .05$. Effect sizes were calculated with partial η^2 and interpreted according to the guidelines by Cohen (1988).

Types of Scores

Several scores were used. First, summed scores on the three pragmatic subdomains were used for the pragmatic language outcomes. Second, a criterion score was used to assess if the total pragmatic score is appropriate for the child's chronological age (e.g., children between the ages of 3.5-4.5 years are expected to have a raw total score of at least 67). Children where then classified as having 'met' or 'not met' age expectations. This age-criterion is provided for the American version of the CELF-P pragmatics profile and to evaluate if the same age-criterion scores could be used in the European sample, the total CELF-P pragmatic scores were compared between the research sites (USA vs NL/BE). As the USA group was younger, age was included in this analysis. No significant differences were found, $F(1,69) = .02$, $p = .882$, partial $\eta^2 < .01$, therefore the age-criterion scores were used in the European sample as well. Third, to compare

children with and without language difficulties in the SCT group, raw scores for expressive semantic and receptive semantic skills were converted to normed scores according to the instrument manual. Next normed scores for these subtests were individually converted into z-scores with a psychometric conversion table for neuropsychological tests (Lezak et al., 2004). Children were considered as having a 'language impairment' if they had a z-score of -1.25 on the receptive (PPVT) and/or expressive (CELF-P EV) structural language task(s); a deviation of 1.25 SD or more below the mean on either receptive or expressive language is often specified as a *specific language impairment* in the literature (Tomblin et al., 1996). Lastly, a 'structural language score' was calculated by averaging the child's converted z-scores on the PPVT, CELF-P EV, and CELF-P SS. At least two of the three scores had to be available in order for the 'structural language score' to be calculated.

Covariates

As we used raw scores, average age of the groups and the age distribution per group was assessed with t-tests and Kolmogorov-Smirnov Z tests respectively. If there was a significant age difference and/or significant difference in the age distribution, age was included in the analysis as covariate.

As there were differences in GIF and SES between the SCT and control group, correlations were calculated between the total pragmatic language score, GIF and SES for the SCT and control group separately. There were significant correlations between the total pragmatic language score and GIF in both groups (SCT: $r = .24$, $p = .050$; Control: $r = .32$, $p = .006$), but no significant correlations between the total pragmatic language score and SES in either group (SCT: $r = .20$, $p = .095$; Control: $r = .04$, $p = .756$). For that reason, only GIF was included as covariate in analyses comparing the SCT and control group.

Analyses

Group Differences SCT versus Controls

Multivariate analysis of covariance (MANCOVA) was used to compare pragmatic language (i.e., nonverbal communication; requesting, giving, and responding to information; conversational routines) outcomes between the SCT and control group. As specific SCT karyotype, time of diagnosis, and ascertainment bias could be relevant for the interpretation of the SCT versus control group results, the impact of these factors was explored with MANCOVA.

First, regarding karyotype specific outcomes, as there were no significant differences between boys and girls in the control group on pragmatic language outcomes (p ranged from .064 to .220), sex dependent effects were also not expected in the SCT group, therefore the three SCT karyotypes (XXX, XXY, XYY) were compared directly. There were no significant differences between the three SCT karyotypes on average age ($p = .605$) or distribution of ascertainment bias ($\chi^2 = 5.59$, $p = .242$), therefore only GIF was included as a covariate in this analysis. There was no significant multivariate effect for SCT karyotype after controlling for GIF, Wilk's Lambda = .88, $F(6,122) = 1.30$, $p = .263$, partial $\eta^2 = .06$, indicating that pragmatic language outcomes are comparable across karyotypes. Second, regarding time of diagnosis, children with a prenatal diagnosis were significantly younger than children with a postnatal diagnosis ($p = .024$), therefore age was included in the analysis as a covariate in addition to GIF. There was no significant multivariate effect for time of diagnosis after controlling for age and GIF, Wilk's Lambda = .98, $F(3,61) = .51$, $p = .675$, partial $\eta^2 = .03$, indicating that pragmatic

language outcomes are comparable between children with a prenatal or postnatal diagnosis. Lastly, regarding ascertainment bias, there were no differences between the three ascertainment groups (i.e., prospective follow-up, information seeking parents, or clinically referred cases) in average age (p = .660), therefore only GIF was included as a covariate in this analysis. There was no significant multivariate effect for ascertainment bias after controlling for GIF, Wilk's Lambda = .84, $F(6,122) = 1.85$, p = .096, partial η^2 = .08, indicating that pragmatic language outcomes are comparable between the three ascertainment bias groups. For each of these factors, the estimated marginal means per pragmatic subdomain can be found in Table 3.

As no effects were found of karyotype, time of diagnosis, or ascertainment bias, these factors were not included in the subsequent SCT versus control group analyses. In addition, there were no differences in average age (p = .138) or age distributions (p = .137) between the SCT and control group, therefore age was not included as a covariate in this analysis. Due to the significant correlations between GIF and pragmatic language, GIF was included as a covariate in analyses comparing the SCT and control group.

Table 3. Pragmatic language abilities as measured with the Clinical Evaluation of Language Fundamentals Preschool Pragmatics Profile: Effects of karyotype, time of diagnosis and ascertainment bias

	SCT karyotype				Time of Diagnosis[a]			Ascertainment Bias[b]			
	XXX	**XXY**	**XYY**	***p***	**Pre**	**Post**	***p***	**A**	**B**	**C**	***p***
N	26	27	14		39	28		26	27	14	
Nonverbal communication	23.54 (.71)	24.42 (.70)	21.56 (.97)	.066	23.59 (.60)	23.32 (.72)	.784	23.30 (.71)	23.38 (.85)	23.85 (.87)	.875
Requesting, giving, and responding to information	19.52 (.73)	20.75 (.72)	18.39 (.96)	.157	19.56 (.57)	20.08 (.68)	.577	20.26 (.70)	19.26 (.84)	18.84 (.85)	.168
Conversational routines	34.16 (1.07)	34.74 (1.06)	30.92 (1.46)	.102	33.44 (.80)	34.10 (.95)	.604	34.38 (1.07)	32.95 (1.27)	33.38 (1.29)	.682

[a] Pre = prenatal, post = postnatal

[b] A = Active prospective follow-up, B = Information seeking parents, C = Clinically referred

Note: Scores represent estimated marginal means (SE) and are co-varied for global level of intellectual functioning (SCT comparisons and ascertainment bias) or global level of intellectual functioning and age (time of diagnosis); higher scores denote better pragmatic skills (raw scores)

Associations with Structural Language

To assess if difficulties with pragmatic language were associated with structural language impairments, three groups were compared: SCT with structural language impairment, SCT without structural language impairment, and controls. See 'types of scores' for our definition of language impairment. As there were two children (1 SCT and 1 control) without a score on either the expressive or receptive structural language task, data from these children was discarded from this analysis. There was no difference in the distribution of SCT karyotypes between the SCT with language impairment and without language impairment, χ^2 = .97, p = .617. There was a significant difference in average age between the three groups (p = .039), therefore, age was included as a covariate.

Clinical Classification

With frequencies and a Chi-square test, the classification of children who did and did not meet the age-criterion was compared between the SCT and control group. With odds ratio, the risk of having a 'clinical score' (i.e., not meeting the age-criterion) was assessed.

Developmental Stability

To test is possible SCT versus control differences on pragmatic language are stable across ages, a PROCESS moderation analysis (Hayes, 2017) was used. Research group (SCT versus controls) was included as predictor, age as moderator, and pragmatic total score as dependent

variable. First, the research group x age interaction was assessed. In case of a nonsignificant interaction effect, a linear hierarchical regression analysis followed to assess the effect of research group (step 1) and to assess the effect of age on top of research group (step 2; method = Enter). If including age improved the initial model, the results from the second model were interpreted.

Predictive Value of Structural and Pragmatic Language Abilities on Behavioral Outcomes

Linear hierarchical regression analyses (Enter method) were used to assess the predictive value of structural and pragmatic language abilities on behavioral outcomes (i.e., ASQ social-emotional problems and CBCL-DSM scales; affective, anxiety, pervasive developmental, attention deficit, and oppositional defiant problems) one year later. For each behavioral outcome separately, structural language outcome was added to the model in the first step, and pragmatic language outcome in the second step (enter method). When including pragmatic language in the second model resulted in an improvement with respect to the first model (significant F change < .05), the model including both structural and pragmatic language was interpreted and reported. Multicollinearity was assessed with the variance inflation factor (VIF). VIF values below 10 were deemed acceptable (Meyers et al., 2006). Part correlations were used as an indication of the percentage of variance accounted for uniquely by each predictor.

Results

Pragmatic Language: SCT versus Controls

There was a significant multivariate effect for research group after controlling for GIF, Wilk's Lambda = .89, $F(3,133) = 5.53$, $p = .001$, partial $\eta^2 = .11$, indicating a moderate to large effect. Univariate effects showed significantly lower scores in the SCT group on all three subdomains, with effect sizes indicating small to medium effects for nonverbal communication and conversational routines and a moderate to large effect for requesting, giving, and responding to information. Univariate outcomes per subdomain can be found in Table 4.

Within the SCT group, 25% of the children did not meet their age-criterion (18 out of 72 children), whereas in the control group 1.4% of the children did not meet their age-criterion (1 out of 71 children). A Chi-square test indicated that that the distribution between SCT children and the control group was significantly different, $\chi^2 = 17.27$, $p < .001$. Odds ratio indicated that the risk of a 'clinical score' (i.e., not meeting the age-criterion) was 23 times higher in the SCT group compared to the control group.

Table 4. Pragmatic language abilities as measured with the Clinical Evaluation of Language Fundamentals Preschool Pragmatics Profile: SCT versus controls

		SCT versus Control		
	SCT	**Control**	***p***	**Partial η^2**
N	67	71		
Nonverbal communication	23.68 (.41)	25.11 (.40)	.016	.04
Requesting, giving, and responding to information	20.11 (.44)	22.62 (.43)	< .001	.10
Conversational routines	34.41 (.68)	36.59 (.66)	.028	.04

Note: Scores represent estimated marginal means (SE) and are co-varied for global level of intellectual functioning; higher scores denote better pragmatic skills (raw scores)

Pragmatic Language: Associations with Language Impairment

There was a statistically significant multivariate effect of group (SCT with structural language impairment, SCT without structural language impairment, control) after controlling for age, Wilk's Lambda = .74, $F(6,270) = 7.45$, $p < .001$, partial $\eta^2 = .14$, indicating a large effect.

Univariate effects showed significant differences between the three groups for all three subdomains, with effect sizes indicating a moderate to large effect for nonverbal communication and large effects for requesting, giving, and responding to information and conversational routines. Significant univariate effects were further explored with pairwise comparisons based on estimated means. For the subdomains nonverbal communication and requesting, giving, and responding to information, children with SCT regardless of structural language abilities had lower outcomes than controls, with no differences between the SCT group with and without language impairment. For the subdomain conversational routines, children with SCT regardless of structural language abilities had lower outcomes than the control group, but in addition, children with SCT and with structural language impairment also had lower scores than children with SCT without language impairment (p = .013). Estimated marginal means and pairwise comparisons can be found in Table 5.

Table 5. Pragmatic language abilities as measured with the Clinical Evaluation of Language Fundamentals Preschool Pragmatics Profile: Associations with language impairment

	SCT *with* language impairment (SCT+)	**SCT *without* language impairment (SCT-)**	**Controls (C)**	***p***	**partial η^2**	**Pairwise comparisons**
N	19	52	70			
Nonverbal communication	23.19 (.76)	23.43 (.45)	25.30 (.39)	.003	.08	SCT+ = SCT- < C
Requesting, giving, and responding to information	18.28 (.80)	20.03 (.48)	23.13 (.41)	<.001	.23	SCT+ = SCT- < C
Conversational routines	30.73 (1.22)	34.31 (.72)	37.66 (.63)	<.001	.18	SCT+ < SCT- < C

Note: Scores represent estimated marginal means (SE) and are co-varied for age; higher scores denote better pragmatic skills (raw scores)

Pragmatic Language: Developmental Stability

The PROCESS analysis did not yield a significant research group (SCT vs controls) x age interaction, p = .989. The inclusion of research group as predictor in the linear regression analysis resulted in a significant model, $F(1,141) = 24.02$, $p < .001$. The addition of age significantly improved the model, $F(2,140) = 17.02$, $p < .001$ ($R^2_{adjusted}$ = .18, significance F change = .004). These results indicate that in both groups pragmatic language scores increase with age, and that children in the control group had higher pragmatic scores than children in the SCT group across age-bins. A visualization of results can be found in Figure 1.

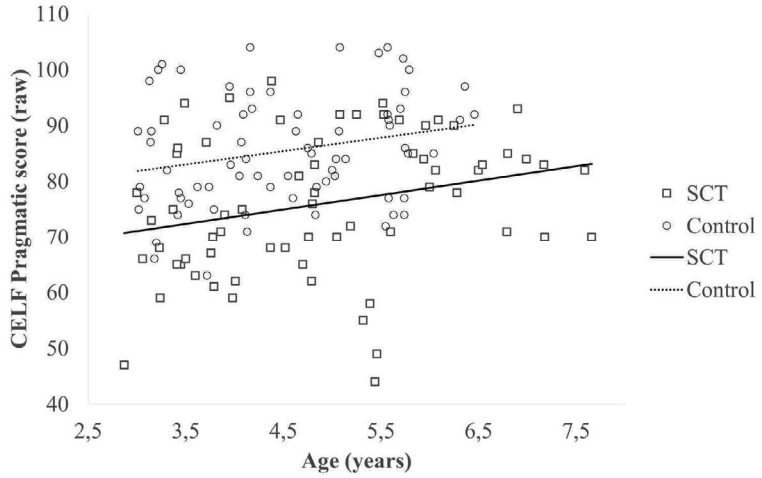

Figure 1. Pragmatic language abilities in the SCT and control groups at different ages (cross-sectional; N_{sct} = 72, $N_{control}$ = 71)

Predictive Value of Structural and Pragmatic Language on Behavioral Outcomes One Year Later

For all outcomes, results for each predictor included in the final model are presented in Table 6. A visualization of results can be found in Figure 2.

Table 6. Predictive value of structural language and pragmatic language on behavioral problems one year later in children with SCT

	Total Model			Structural			Pragmatic		
	R	**R^2**	***p***	***β***	**Part corr.**	**VAF**	***β***	**Part corr.**	**VAF**
Anxiety	.23	.05	.308	-.08	-.08		-.21	-.20	
Affective	.45	.20	.009	-.21	-.21		-36*	-.35	12.5%
Oppositional defiant	.41	.17	.021	.02	.01		-.41**	-.41	16.5%
Attention deficit	.63	.39	<.001	-.29*	-.29	8.1%	-.51***	-.50	24.7%
Pervasive developmental	.81	.66	<.001	-53***	-.52	26.7%	-.53***	-.53	27.6%
Social-emotional	.73	.53	<.001	-.26*	-.25	6.5%	-.64***	-.63	39.1%

* p < .05; ** p <.01; *** p < .001
Abbreviations Part corr. = part correlation; VAF = unique variance accounted for by this variable
Note: N = 48 for anxiety, affective, oppositional defiant, attention deficit, and social emotional. N = 29 for pervasive developmental

Unique Predictive Value of Pragmatic Language

For two of the behavioral outcomes, only pragmatic language was a significant predictor in the model. Taken together, results indicated that more affective problems and more oppositional defiant problems one year later were predicted by more pragmatic language difficulties.

First, structural and pragmatic language together explained 19.9% of the variance in longitudinal affective problems, $F(2,42) = 5.23$, $p = .009$, with pragmatic language uniquely accounting for 12.5% of the variance ($p = .014$). Structural language was not a significant predictor once pragmatic language was taken into account ($p = .144$), nor was it a significant predictor on its own ($p = .070$)

Second, structural and pragmatic language together explained 16.8% of the variance in longitudinal oppositional defiant problems, $F(2,42) = 4.24$, $p = .021$, with pragmatic language uniquely accounting for 16.5% of the variance ($p = .006$). Structural language was not a

significant predictor once pragmatic language was taken into account (p = .919), nor was it a significant predictor on its own (p = .704)

Combined Predictive Value of Pragmatic Language and Structural Language.

For three of the behavioral outcomes, both structural language and pragmatic language were significant predictors in the model. Taken together, results indicated that more attention deficit problems, more pervasive developmental problems, and more social-emotional problems one year later were predicted by more pragmatic language difficulties and more structural language difficulties.

First, structural and pragmatic language together explained 39.1% of the variance in longitudinal attention deficit problems, $F(2,42) = 13.49$, $p < .001$. Pragmatic language (p <.001) uniquely accounted for 24.7% of the variance and structural language (p = .022) uniquely accounted for 8.1% of the variance in attention deficit problems.

Second, structural and pragmatic language together explained 66.0% of the variance in longitudinal pervasive developmental problems, $F(2,25) = 24.25$, $p < .001$. Pragmatic language ($p < .001$) uniquely accounted for 27.6% of the variance and structural language ($p < .001$), uniquely accounted for 26.7% of the variance in pervasive developmental problems.

Third, structural and pragmatic language together explained 52.8% of the variance in social-emotional problems, $F(2,42) = 23.50$, $p < .001$. Pragmatic language ($p < .001$), uniquely accounted for 39.1% of the variance and structural language (p = .021), accounted for 6.5% of the variance in social-emotional problems

No Predictive Value of Pragmatic Language and Structural Language

For anxiety problems regression results did not yield a significant model, $F(2,42) = 1.21$, p = .308. Structural and pragmatic language were not predictive of longitudinal anxiety problems.

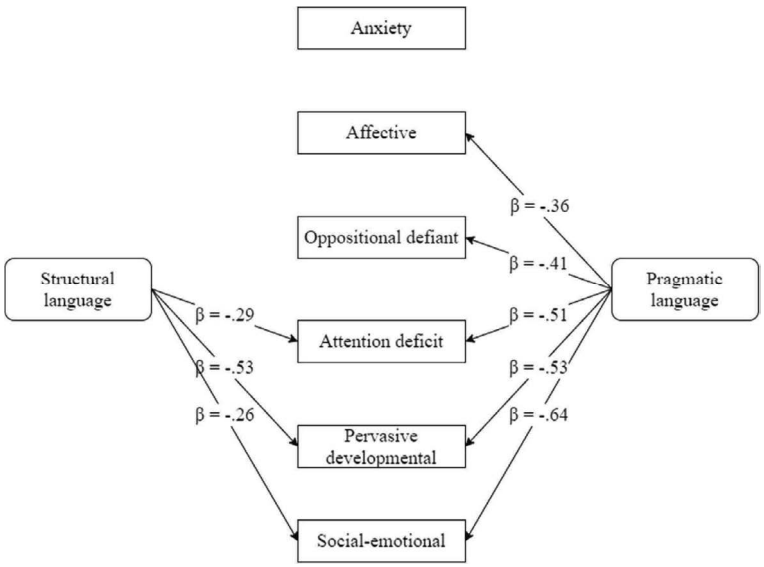

Figure 2. Predictive value of structural language and pragmatic language on behavioral outcomes one year later in children with SCT (N_{range} = 29 -48)

Predictive Value of Structural and Pragmatic Language on Behavioral Outcomes One Year Later: Control group

Structural and/or pragmatic language were predictive for behavioral outcomes one year later in the control group as well. Structural language on its own was predictive for both oppositional defiant problems and attention deficit problems one year later, uniquely accounting for 9.1% and 15.5% of the variance respectively. Pragmatic language on its own was predictive for social-emotional problems one year later, uniquely accounting for 10.4% of the variance. No predictive value of structural or pragmatic language was found for anxiety, affective, and pervasive developmental problems one year later. For all outcomes, results for each predictor included in the model are presented in Table 7.

Table 7. Predictive value of structural language and pragmatic language on behavioral problems one year later in the control group

	Total Model			Structural			Pragmatic		
	R	**R^2**	***p***	***β***	**Part corr.**	**VAF**	***β***	**Part corr.**	**VAF**
Anxiety	.26	.07	.379	-.17	-.16		-.14	-.13	
Affective	.19	.04	.157	-.08	-.07		-.15	-.14	
Oppositional defiant[a]	.30	.09	.021	-.30*	-.30	9.1%	-	-	
Attention deficit[a]	.39	.16	.002	-.39**	-.39	15.5%	-	-	
Pervasive developmental	.23	.05	.404	-.12	-.11		-.17	-.16	
Social-emotional	.34	.12	.031	-.01	-.01		-.34*	-.32	10.4%

* $p < .05$; ** $p < .01$; *** $p < .001$

[a] As adding pragmatic language did not improve the model, the model that includes only structural language was reported and interpreted.

Abbreviations Part corr. = part correlation; VAF = unique variance accounted for by this variable

Note: N = 58 for anxiety, affective, oppositional defiant, attention deficit, and social emotional. N = 36 for pervasive developmental

Discussion

The aim of this study was two-fold. First, to determine if children with SCT also have compromised pragmatic language abilities; in other words, do children with SCT have communication deficits beyond structural language difficulties. Second, to determine if pragmatic language, above and beyond structural language, is predictive of neurobehavioral outcomes in later development.

With regard to the first aim, we addressed several questions. First, regarding average pragmatic language abilities, children in the SCT group had lower average scores on all included domains. These differences were not driven by SCT specific characteristics (i.e., karyotype, time of diagnosis, ascertainment bias). In addition, 25% of the children did not meet age expectations. Odds ratio indicates that the risk of having inadequate pragmatic language abilities is 23 times higher in the SCT group, compared to the control group. As the present study is one of the first studies to investigate pragmatic language abilities in children with SCT, it is important that findings of this study are replicated in other cohorts as our findings indicate that pragmatic language is a vulnerable domain for children with SCT. Pragmatic language abilities include nonverbal communication abilities, ability to request, give and respond to information, and the ability to engage in conversational routines. When nonverbal communication abilities are affected, this is possibly not only associated to someone's ability to *use* nonverbal communication to send a message, but also to someone's ability to *understand* nonverbal communication. When the ability to request, give, or respond to information or the ability to engage in conversational routines is affected, this could go together with someone's ability to use language for different purposes or with one's ability to follow the unspoken rules of conversation. These results show that in addition to structural language difficulties, pragmatic language can also be affected in this population. We suggest that these pragmatic language difficulties should be considered as part of a broader communication deficit. This is

in line with findings that illustrate other difficulties in individuals with SCT that are part of or related to social communication; such as difficulties with understanding someone else's perspective (i.e., Theory of Mind; Bouw, Swaab, Tartaglia, & van Rijn, 2021; van Rijn, Stockmann, van Buggenhout, et al., 2014), the ability to adapt adequately to the situation, and further language and communication development (Matthews et al., 2018). Individuals with SCT are often described as shy, timid, and withdrawn (for a review see Leggett et al., 2010). In addition, social difficulties, such as difficulties with reading social signals such as facial emotional recognition (Bouw, Swaab, Tartaglia, Cordeiro, et al., 2021; van Rijn et al., 2018; van Rijn, Stockmann, van Buggenhout, et al., 2014) and tone of voice (Van Rijn et al., 2007) have been reported. Since pragmatic language abilities are interconnected with social skills and emotional understanding (Parsons et al., 2017), it is likely that these social difficulties in individuals with SCT are the result of a global communication deficit.

Second, we addressed the question whether pragmatic language problems were associated with language difficulties. Results indicated that not only children with language impairments experience difficulties with the social use of language, but rather that pragmatic difficulties are a more common characteristic within the SCT group. For nonverbal communication and requesting, giving, or responding to information, children in the SCT group on average had lower abilities than children in the control group, regardless of the presence of a language impairment. Children with SCT showed more challenges with engaging in conversational routines than controls, regardless of the presence of a language impairment, but these skills appeared to be even more compromised in children with SCT *and* language impairment. Taken together, these results show that pragmatic language abilities are a vulnerable domain in the SCT group, and that some pragmatic language abilities can be more pronounced when they co-occur with structural language abilities.

Third, looking at age-effects within this cross-sectional sample, results show that pragmatic language abilities continue to develop in both children with SCT and controls. However, across all ages, children in the SCT group have lower outcomes than controls. This suggests that, although pragmatic language abilities improve in children with SCT and that children with SCT do not necessarily deviate more from the norm when they get older, pragmatic language difficulties can be considered persistent in the SCT group (Bouw, Swaab, Tartaglia, & van Rijn, 2021; van Rijn, Stockmann, van Buggenhout, et al., 2014).

Regarding our second aim – the predictive value of structural and pragmatic language on later behavior outcomes – our findings illustrate the relevance of language skills for a variety of neurobehavioral outcomes in both children with SCT as well as controls. In the SCT group, pragmatic language was predictive of a broader variety of behavioral outcomes than structural language, and for some behavioral outcomes the ability to use language as a social tool was the sole predictor. Thus, pragmatic abilities are important skills to consider in children with SCT, uniquely contributing to behavioral problems when also taking structural language into account. Although structural and pragmatic language were also predictive of behavioral outcomes in the control group, the pattern of results differed from the results in the SCT group. In a study with 4-year-old children from a community sample, children who met the criteria for pragmatic language impairment and thus had lower pragmatic scores showed more behavioral problems than their peers without pragmatic language impairment (Ketelaars et al., 2010). This finding is in line with the current paper and the current paper adds to this, by studying a group of children who biologically are at increased risk for language difficulties and unfavorable

behavioral outcomes. The striking finding that early social-communicative abilities explain a large part of the variance in neurobehavioral outcomes highlights the importance of early monitoring and the need for early support and intervention opportunities.

The results of this study have important clinical implications; they illustrate that early social-communicative abilities can be an important marker to identify children with SCT who are at risk for unfavorable outcomes at an early age, and for outcomes that are possibly also related to the risk for more severe psychopathology in later life. Thus, it is important to not only include structural language abilities, but also pragmatic language abilities in routine monitoring; and to look at the broader communication abilities of children with SCT. In addition, this shows that pragmatic language might be an important target for interventions as it is possible that supporting the development of pragmatic language could also have positive effects on behavioral outcomes. Lastly, it should be noted that although some children appear to be severely affected, other children are less affected or do not have notable differences from peers. In order to understand which children are vulnerable, it is important to gain more knowledge on the development of pragmatic language in young children with SCT.

The presence of an extra X or Y chromosome impacts the development of the brain (Raznahan et al., 2016); possibly including structures that are important for social communication. Although causality is not implied, the fact that difficulties with pragmatic language occur at an early age could be an important signal for deviant brain maturation. As SCT can be diagnosed prenatally, the impact of early mechanisms of developmental risk can already be studied from birth, providing the unique opportunity to study the earliest forms of communicative development in a homogenous group with a clear genetic cause. In contrast, studying groups of children with behavioral diagnoses, such as specific language impairment, limits this opportunity, as these children often form a heterogeneous group and children will not be identified until problems in daily functioning have presented themselves. In addition, as the results of this study illustrate the impact of the X and Y chromosome on pragmatic language outcomes, genes on these chromosomes could serve as possible candidate genes to explain variability in outcomes in the general population. In sum, studying communication skills in young children with SCT could give valuable insight in underlying mechanisms and developmental pathways to neurodevelopmental impact and psychopathology, and therefore increase our understanding of development and developmental risk, not only in the SCT population, but in the general population as well.

Within the present study, we were able to include a relatively large group of children at a young age. Due to the longitudinal design, we were able to make predictions in behavioral outcomes over time, although some data was missing, primarily due to the worldwide COVID-19 pandemic. There were some limitations in this study. First, only children aged 3 years or older were included, whereas social interaction and communication can already be assessed in younger children. It is important to learn more about the social communication abilities in children who are followed from birth, to pinpoint if difficulties in social communication can already be detected from birth or if they occur as a result of the development of the brain. Second, with this international sample, we were able to include a large cohort of children. Although our findings did not indicate differences in children with SCT from the USA and from the Dutch speaking parts of Western Europe, future studies could further explore cultural differences. In addition, other factors that could possibly play a mediating role in pragmatic language outcomes could be explored further. In our study, there were differences in SES and

GIF between the SCT and control group. This difference was accounted for by including GIF as covariate in the analyses. However, it should be noted that by including GIF as a covariate, shared covariance between GIF and pragmatic language is filtered out. This possibly could have led to an underestimation of pragmatic language difficulties. SES however, although different between the SCT and control group, did not appear to play a role in pragmatic abilities, as illustrated by the non-significant correlation. Also, we cannot rule out that some children may have received some form of care as usual intervention, targeting language and/or communication skills within the timeframe of the study, which could possibly impact the studied associations with later behavioral outcomes. Future studies should further look into the contribution of both environmental factors (e.g., the 'language-richness' of the environment) and interpersonal factors (e.g., services received, including hormonal treatments in the XXY group). Third, the composite structural language score in this study was based on children's expressive semantic skills, receptive semantic skills, and syntactic abilities. There is more to language and communication than the included parameters in this study and future studies are encouraged to add to the growing body of literature examining the development of language and communication skills and how these skills are related to behavioral outcomes in children with SCT. A fourth limitation of this study is the use of a parent questionnaire to assess pragmatic language outcomes. Pragmatic language can also be assessed with performance-driven measures, participant transcript, or semi-naturalistic measures. Future studies are encouraged to incorporate a combination of these measures to gain a better understanding of the reach of pragmatic abilities in children with SCT. In addition, to avoid the possibility of shared-method variance, it would be ideal to use the same 'informant' for all predictive variables (i.e., use parent questionnaires or child performance tasks for both structural and pragmatic outcomes), which is a limitation of the design of the current study. Finally, while studies designed to analyze predictors of later outcomes such as this study are unique, it is important that future studies investigate the developmental trajectory of pragmatic language, behavioral outcomes, and the predictive value of language outcomes for behavioral outcomes across a longer time span. Within this study, we identified functions on the language and communication domain as building blocks for later behavioral outcomes. It is important to further explore other neurocognitive domains, for example social cognitive functioning or executive functioning, to further unravel which mechanisms underly adverse behavioral outcomes. It should be noted that the development of children is dynamic; child characteristics interact with behavioral outcomes. For example, a child with language difficulties may socially isolate, resulting in less language learning experiences, which eventually could lead to worse language outcomes. It is important to take this dynamic interaction into account. Taken together, future studies should look into the social communicative abilities of children under the age of three, investigate possible mediating factors, and project outcomes over a longer time period.

To conclude, our data suggest that children with SCT are at risk for communication deficits that extend beyond structural language abilities, including difficulties to use language in a social context. The relevance of early assessment of a broad spectrum of communication skills in addition to structural language skills is illustrated by the fact that pragmatic deficits are not limited to children with structural language deficits but can be identified in those without structural language deficits as well. Most importantly, the social use of language seems to have stronger predictive value than structural language abilities for a broad range of neurobehavioral outcomes one year later. Thus, it is important to monitor not only structural language

development, but also pragmatic language development in young children with SCT, since pragmatic language development, can serve as a marker for children who are at risk of developing behavioral and social-emotional problems.

References

Achenbach, T., & Ruffle, T. (2000). The child behavior checklist and related forms for assessing behavioral/emotional problems and competencies. *Pediatrics in Review*, *21*, 265-271. https://doi.org/10.1542/pir.21-8-265

Asada, K., Tomiwa, K., Okada, M., & Itakura, S. (2010). Fluent language with impaired pragmatics in children with Williams syndrome. *Journal of Neurolinguistics*, *23*(6), 540-552. https://doi.org/10.1016/j.jneuroling.2010.04.001

Bishop, D. V. M., Brookman-Byrne, A., Gratton, N., Gray, E., Holt, G., Morgan, L., . . . Thompson, P. A. (2018). Language phenotypes in children with sex chromosome trisomies. *Wellcome Open Research*, *3*, 143. https://doi.org/10.12688/wellcomeopenres.14904.2

Bishop, D. V. M., Jacobs, P. A., Lachlan, K., Wellesley, D., Barnicoat, A., Boyd, P. A., . . . Scerif, G. (2011). Autism, language and communication in children with sex chromosome trisomies. *Archives of Disease in Childhood*, *96*(10), 954-959. https://doi.org/10.1136/adc.2009.179747

Boada, R., Janusz, J., Hutaff-Lee, C., & Tartaglia, N. (2009). The cognitive phenotype in Klinefelter syndrome: A review of the literature including genetic and hormonal factors. *Developmental Disabilities Research Reviews*, *15*(4), 284-294. https://doi.org/10.1002/ddrr.83

Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevelance of Klinefelter syndrome: A national registry study. *The Journal of Clinical Endocrinology & Metabolism*, *88*(2), 622-626. https://doi.org/10.1210/jc.2002-021491

Bouw, N., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2021). Social attention and affect recognition skills in very young children with sex chromosome trisomies. An eyetracking study.

Bouw, N., Swaab, H., Tartaglia, N., & Van Rijn, S. (2021). The impact of sex chromosome trisomies (XXX, XXY, XYY) on early social cognition: Social orienting, joint attention and theory of mind. *Archives of Clinical Neuropsychology*, Article acab042. https://doi.org/10.1093/arclin/acab042

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Lawrence Erlbaum Associates.

Cordeiro, L., Tartaglia, N., Roeltgen, D., & Ross, J. (2012). Social deficits in male children and adolescents with sex chromosome aneuploidy: A comparison of XXY, XYY, and XXYY syndromes. *Research in Developmental Disabilities*, *33*(4), 1254-1263. https://doi.org/10.1016/j.ridd.2012.02.013

Dunn, L. M., & Dunn, L. M. (1997). *Peabody Picture Vocabulary Test - Third Edition Manual*. American Guidance Service

Dunn, L. M., & Dunn, L. M. (2005). *Peabody Picture Vocabulary Test-III-NL. Handleiding* (L. Schlichting, Trans.). Harcourt Test Publishers.

Gallagher, T. M. (1999). Interrelationships among children's language, behavior, and emotional problems. *Topics in Language Disorders*, *19*(2), 1-15. https://doi.org/10.1097/00011363-199902000-00003

Gilmour, J., Hill, B., Place, M., & Skuse, D. H. (2004). Social communication deficits in conduct disorder: A clinical and community survey. *Journal of Child Psychology and Psychiatry*, *45*(5), 967-978. https://doi.org/10.1111/j.1469-7610.2004.t01-1-00289.x

Gremillion, M. L., & Martel, M. M. (2014). Merely misunderstood? Receptive, expressive, and pragmatic language in young children with disruptive behavior disorders. *Journal of Clinical Child & Adolescent Psychology*, *43*(5), 765-776. https://doi.org/10.1080/15374416.2013.822306

Groth, K. A., Skakkebaek, A., Høst, C., Gravholt, C. H., & Bojesen, A. (2013). Klinefelter syndrome - a clinical update. *The Journal of Clinical Endocrinology & Metabolism*, *98*(1), 20-30. https://doi.org/10.1210/jc.2012-2382

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach* (Second edition ed.). Guilford Press.

Ketelaars, M. P., Cuperus, J., Jansonius, K., & Verhoeven, L. (2010). Pragmatic language impairment and associated behavioural problems. *International Journal of Language & Communication Disorders*, *45*(2), 204-214. https://doi.org/10.3109/13682820902863090

Lee, N. R., Wallace, G. L., Adeyemi, E. I., Lopez, K. C., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2012). Dosage effects of X and Y chromosomes on language and social functioning in children with supernumerary sex chromosome aneuploidies: Implications for idiopathic language impairment and autism spectrum disorders. *Journal of Child Psychology and Psychiatry*, *53*(10), 1072-1081. https://doi.org/10.1111/j.1469-7610.2012.02573.x

Leggett, V., Jacobs, P., Nation, K., Scerif, G., & Bishop, D. V. (2010). Neurocognitive outcomes of individuals with a sex chromosome trisomy: XXX, XYY, or XXY: A systematic review. *Developmental Medicine & Child Neurology*, *52*(2), 119-129. https://doi.org/10.1111/j.1469-8749.2009.03545.x

Lenroot, R. K., Blumenthal, J. D., Wallace, G. L., Clasen, L. S., Lee, N. R., & Giedd, J. N. (2014). A case-control study of brain structure and behavioral characteristics in 47,XXX syndrome. *Genes Brain and Behavior*, *13*(8), 841-849. https://doi.org/10.1111/gbb.12180

Lezak, M. D., Howieson, D., Loring, D., & Hannay, H. (2004). *Neuropsychological assessment* (4th ed.). Oxford University Press.

Matthews, D., Biney, H., & Abbot-Smith, K. (2018). Individual differences in children's pragmatic ability: A review of associations with formal language, social cognition, and executive functions. *Language Learning and Development*, *14*(3), 186-223. https://doi.org/10.1080/15475441.2018.1455584

Melogno, S., Pinto, M. A., Badolato, F., Sist, E., Esposito, A., Orsolini, M., & Tarani, L. (2019). High-level language competencies and Theory of Mind in a group of children with Klinefelter syndrome. *American Journal of Medical Genetics Part A*, *179*(2), 183-189. https://doi.org/10.1002/ajmg.a.12

Meyers, L. S., Gamst, G., & Guarino, A. J. (2006). *Applied multivariate research: Design and interpretation*. Sage Publications, Inc.

Miranda, A., Berenguer, C., Rosello, B., & Baixauli, I. (2020). Relationships between the social communication questionnaire and pragmatic language, socialization skills, and behavioral problems in children with autism spectrum disorders. *Applied Neuropsychology: Child*, *9*(2), 141-152. https://doi.org/10.1080/21622965.2018.1550403

Morris, J. K., Alberman, E., Scott, C., & Jacobs, P. (2008). Is the prevalence of Klinefelter syndrome increasing? *European Journal of Human Genetics*, *16*, 163-170. https://doi.org/10.1038/sj.ejhg.5201956

Netley, C. T. (1986). Summary overview of behavioural development in individuals with neonatally identified X and Y aneuploidy. In S. G. Ratcliffe & N. Paul (Eds.), *Prospective studies on children with sex chromosome aneuploidy* (Vol. 22, pp. 293-306). Alan R. Liss, Inc.

Parsons, L., Cordier, R., Munro, N., Joosten, A., & Speyer, R. (2017). A systematic review of pragmatic language interventions for children with autism spectrum disorder. *PLoS One*, *12*(4), e0172242. https://doi.org/10.1371/journal.pone.0172242

Raznahan, A., Lee, N. R., Greenstein, D., Wallace, G. L., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2016). Globally divergent but locally convergent X- and Y-chromosome influences on cortical development. *Cerebral Cortex*, *26*, 70-79. https://doi.org/10.1093/cercor/bhu174

Ross, J. L., Roeltgen, D. P., Kushner, H., Zinn, A. R., Reiss, A., Bardsley, M. Z., . . . Tartaglia, N. (2012). Behavioral and social phenotypes in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Pediatrics*, *129*(4), 769-778. https://doi.org/10.1542/peds.2011-0719

Ross, J. L., Roeltgen, D. P., Stefanatos, G., Benecke, R., Zeger, M. P. D., Kushner, H., . . . Zinn, A. R. (2008). Cognitive and motor development during childhood in boys with Klinefelter syndrome. *American Journal of Medical Genetics Part A*, *146A*(6), 708-719. https://doi.org/10.1002/ajmg.a.32232

Ross, J. L., Zeger, M. P., Kushner, H., Zinn, A. R., & Roeltgen, D. P. (2009). An extra X or Y chromosome: contrasting the cognitive and motor phenotypes in childhood in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Developmental Disabilities Research Reviews*, *15*(4), 309-317. https://doi.org/10.1002/ddrr.85

Rovet, J., Netley, C., Bailey, J., Keenan, M., & Stewart, D. (1995). Intelligence and achievement in children with extra X aneuploidy: A longitudinal perspective. *American Journal of Medical Genetics*, *60*, 356-363.

Rovet, J., Netley, C., Keenan, M., Bailey, J., & Stewart, D. (1996). The psychoeducational profile of boys with Klinefelter syndrome. *Journal of Learning Disabilities*, *29*(2), 180-196.

Samango-Sprouse, C., Stapleton, E., Chea, S., Lawson, P., Sadeghin, T., Cappello, C., . . . Van Rijn, S. (2018). International investigation of neurocognitive and behavioral phenotype in 47,XXY (Klinefelter syndrome): Predicting individual differences. *American Journal of Medical Genetics Part A*, *176*(4), 877-885. https://doi.org/10.1002/ajmg.a.38621

Squires, J., Bricker, D., & Twombly, E. (2015). *Ages & stages questionnaires®: Social-emotional, second edition (ASQ®:SE-2): A parent-completed child monitoring system for social-emotional behaviors*. Paul H. Brookes Publishing Co., Inc.

St John, M., Ponchard, C., van Reyk, O., Mei, C., Pigdon, L., Amor, D. J., & Morgan, A. T. (2019). Speech and language in children with Klinefelter syndrome. *Journal of Communication Disorders*, *78*, 84-96. https://doi.org/10.1016/j.jcomdis.2019.02.003

Staikova, E., Gomes, H., Tartter, V., McCabe, A., & Halperin, J. M. (2013). Pragmatic deficits and social impairment in children with ADHD. *Journal of Child Psychology and Psychiatry*, *54*(12), 1275-1283. https://doi.org/10.1111/jcpp.12082

Tartaglia, N. R., Cordeiro, L., Howell, S., Wilson, R., & Janusz, J. (2010). The spectrum of the behavioral phenotype in boys and adolescents 47,XXY (Klinefelter Syndrome). *Pediatric Endocrinology Reviews*, *8*, 151-159.

Tomblin, J. B., Records, N. L., & Zhang, X. (1996). A system for the diagnosis of specific language impairment in kindergarten children. *Journal of Speech and Hearing Research*, *39*, 1284-1294. https://doi.org/10.1044/jshr.3906.1284

Urbanus, E., Swaab, H., Tartaglia, N., Boada, R., & Van Rijn, S. (2021). A cross-sectional study of early language abilities in children with sex chromosome trisomy (XXY, XXX, XYY) aged 1-6 years. *Child Neuropsychology*, *28*(2), 171-196. https://doi/org/10.1080/09297049.2021.1960959

Urbanus, E., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2020). The behavioral profile of children aged 1-5 years with sex chromosome trisomy (47,XXX, 47,XXY, 47,XYY). *American Journal of Medical Genetics part C*, *184C*, 444-455. https://doi.org/10.1002/ajmg.c.31788

Urbanus, E., Van Rijn, S., & Swaab, H. (2019). A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention. *Clinical Genetics*, *97*(1), 156-167. https://doi.org/10.1111/cge.13586

Van Rijn, S. (2019). A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47,XXX, 47,XYY). *Current Opinion in Psychiatry*, *32*(2), 79-84. https://doi.org/10.1097/yco.0000000000000471

Van Rijn, S., Aleman, A., Swaab, H., Krijn, T., Vingerhoets, G., & Kahn, R. S. (2007). What it is said versus how it is said: Comprehension of affective prosody in men with Klinefelter (47,XXY) syndrome. *Journal of the International Neuropsychological Society*, *13*(6), 1065-1070. https://doi.org/10.1017/s1355617707071044

Van Rijn, S., de Sonneville, L., & Swaab, H. (2018). The nature of social cognitive deficits in children and adults with Klinefelter syndrome. *Genes Brain and Behavior*, *17*(6), e12465. https://doi.org/10.1111/gbb.12465

Van Rijn, S., Stockmann, L., Borghgraef, M., Bruining, H., van Ravenswaaij-Arts, C., Govaerts, L., . . . Swaab, H. (2014). The social behavioral phenotype in boys and girls with an extra X chromosome (Klinefelter syndrome and Trisomy X): A comparison with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *44*(2), 310-320. https://doi.org/10.1007/s10803-013-1860-5

Van Rijn, S., Stockmann, L., van Buggenhout, G., van Ravenswaaij-Arts, C., & Swaab, H. (2014). Social cognition and underlying cognitive mechanisms in children with an extra X chromosome: a comparison with autism spectrum disorder. *Genes Brain Behav*, *13*(5), 459-467. https://doi.org/10.1111/gbb.12134

Visootsak, J., & Graham, J. M., Jr. (2009). Social function in multiple X and Y chromosome disorders: XXY, XYY, XXYY, XXXY. *Developmental Disabilities Research Reviews*, *15*(4), 328-332. https://doi.org/10.1002/ddrr.76

Volden, J., Coolican, J., Garon, N., White, J., & Bryson, S. (2009). Brief report: Pragmatic language in autism spectrum disorder: Relationships to measures of ability and disability. *Journal of Autism and Developmental Disorders*, *39*(2), 388-393. https://doi.org/10.1007/s10803-008-0618-y

Wechsler, D. (2002). *WPPSI-III: Technical and interpretative manual* The Psychological Corporation

Wechsler, D., & Naglieri, J. A. (2006). *Wechsler Nonverbal Scale of Ability*. Harcourt Assessment

Wiig, E. H., Secord, W. A., & Semel, E. (2004). *Clinical Evaluation of Language Fundamentals - Preschool (2nd ed.)*. Harcourt Assessment

Wiig, E. H., Secord, W. A., & Semel, E. (2012). *Clinical Evaluation of Language Fundamentals Preschool (CELF-2NL) - Nederlandse Versie* (J. de Jong, Trans.). Pearson Assessment and Information.

Zampini, L., Burla, T., Silibello, G., Capelli, E., Dall'Ara, F., Rigamonti, C., . . . Vizziello, P. G. (2020). Preverbal skills in 8-month-old children with sex chromosome trisomies. *First Language*, *41*(2), 200-217. https://doi.org/10.1177/0142723720962944

Zampini, L., Burla, T., Silibello, G., Dall'Ara, F., Rigamonti, C., Lalatta, F., & Vizziello, P. (2017). Early communicative skills of children with Klinefelter syndrome. *Clinical Linguistics & Phonetics*, *32*(7), 577-586. https://doi.org/10.1080/02699206.2017.1384853

Zampini, L., Draghi, L., Silibello, G., Dall'Ara, F., Rigamonti, C., Suttora, C., . . . Vizziello, P. (2018). Vocal and gestural productions of 24-month-old children with sex chromosome trisomies. *International Journal of Language & Communication Disorders*, *53*(1), 171-181. https://doi.org/10.1111/1460-6984.12334

Supplementary Materials

Correlation Matrices Pragmatic Language Subdomains and Included Variables Regression Analyses: SCT group only

Table A1. Correlations pragmatic language subdomains

	1. Nonverbal communication	2. Requesting, giving, and responding to information
Nonverbal communication	-	
Requesting, giving, and responding to information	.69***	-
Conversational routines	.70***	.80***

* $p < .05$; ** $p < .01$; *** $p < .001$

Table A2. Correlations structural language, total pragmatic score, and behavioral outcomes

	1. Structural language	2. Pragmatic total score	3. Anxiety	4. Affective	5. Oppositional defiant	6. Attention deficit	7. Pervasive developmental
Structural language	-						
Pragmatic total score	.18	-					
Anxiety	-.12	-.22	-				
Affective	-.27	-.40**	.38**	-			
Oppositional defiant	-.06	-.41**	.30*	.63***	-		
Attention deficit	-.38**	-.56***	.30*	.68***	.63***	-	
Pervasive developmental	-.62***	-.63***	.62***	.61***	.33	.46*	-
Social-emotional	-.37*	-.68***	.32*	.59***	.61***	.75***	.64***

* $p < .05$; ** $p < .01$; *** $p < .001$

Chapter 6

Social communication deficit in children with sex chromosome trisomy (XXY, XXX, XYY)?

Social orientation measured with eye tracking and physiological arousal responses during short communicative interaction paradigms

Submitted as:

Urbanus, E., Swaab, H., Tartaglia, N., & Van Rijn, S. Social communication deficit in children with sex chromosome trisomy (XXY, XXX, XYY)? Social orientation measured with eye tracking and physiological arousal responses during short communicative interaction paradigms.

Abstract

The authors studied social orientation with eye-tracking and physiological arousal responses to gain insight in how children (1-7 years) with sex chromosome trisomy (SCT) perceive and respond to communicative interactions. Assessment and recruitment took place in the USA and Western-Europe. Compared to controls (58 girls, 44 boys), children with SCT (33 XXX girls, 50 XXY boys, 24 XYY boys) showed reduced attention to the face and eyes of the on-screen interaction partner and reduced physiological arousal sensitivity in response to direct versus averted gaze. This suggest that children with SCT may experience difficulties with social communication that extend past the well-recognized risk for early language delays. These difficulties may underlie social-behavioral problems and are a promising target for early interventions.

Introduction

Due to a *de novo* error in early cell division, approximately 1:650-1:1000 children is born with an extra X or Y chromosome or sex chromosome trisomy (SCT; Berglund et al., 2019; Groth et al., 2013). An extra X chromosome leads to a 47,XXX karyotype in females or 47,XXY karyotype in males, while an extra Y chromosome in males leads to a 47,XYY karyotype. This high prevalence makes SCT one of the most common genetic disorders in humans (Hong & Reiss, 2014). SCT can be detected before birth, resulting in a relative unique opportunity to study the effects of an extra sex chromosome on neurocognitive and behavioral development from an early age. Genes that are located on both the X and Y chromosomes play an important role in neural development (Raznahan et al., 2016). Subsequently, children with SCT have an increased risk for suboptimal neurodevelopment, with studies reporting higher incidences of neurodevelopmental disorders (for a review see Van Rijn, 2019) and neurocognitive difficulties (for a review see Urbanus et al., 2019) compared to population samples.

Difficulties with language have frequently been reported in individuals with SCT. Studies on language outcomes have shown compromised language abilities in children as young as 8 months old (Zampini et al., 2020). Difficulties with language can already be noted in the preverbal stage (e.g., use of communicative gestures), and appear to cover a wide range of language abilities, including but not limited to semantic language, syntax, and pragmatic language (Bishop et al., 2011; Ross et al., 2008; Ross et al., 2009; St John et al., 2019; Urbanus, Swaab, Tartaglia, Boada, et al., 2021; Urbanus, Swaab, Tartaglia, Stumpel, et al., 2021; Zampini et al., 2020; Zampini et al., 2017; Zampini et al., 2018). As these language difficulties can already be apparent at a very young age and multiple language abilities appear to be affected, it is likely that these difficulties are anchored in early brain maturation. Considering the importance of language in social communication, it is thought that language difficulties may help explain the social behavioral difficulties that have been observed in the SCT population. However, there is more to social communication that language alone. It is important to gain more knowledge on the broader communicative skills of children with SCT. Assessments to pinpoint strengths and weaknesses in the overall communicative domain will result in knowledge that could be used for early detection of the broad spectrum of verbal and nonverbal communicative problems and ultimately for development of tailored and comprehensive intervention programs that focus on the broad spectrum of communication skills.

Preferential looking at faces and face-like stimuli over non-social stimuli is a natural phenomenon in infants and children. This preference to faces and face-like stimuli, or social attention, may facilitate communicative engagement (Frazier Norbury et al., 2009). Social attention can be divided into three constructs (Dawson et al., 2004): Social orienting (i.e., the ability to direct one's attention to another person, spontaneously or when requested; Guillon et al., 2014), joint attention (i.e., the capacity to share attention with others in a coordinated way; Nation & Penny, 2008) and attending to distress and emotions of others (i.e., the ability to understand and communicate about emotional states and desires; Sigman et al., 1992). These three constructs are crucial in early development; children with impaired social attention may experience difficulties with understanding the social world around them, which may result in compromised development of adaptive social behaviors. In addition, social attention plays an important role in language acquisition and development (Mundy & Neal, 2000). In this study, the focus will be on social orientation.

The ability to orient to the face of another individual can help children learn about speech sounds, facilitating early vocabulary learning (Hillairet de Boisferon et al., 2018). Also, the ability to orientate to relevant aspects of a social scene can reflect a child's sensitivity to pick up relevant (nonverbal) communicative cues. Focus on the mouth while looking at someone who is speaking indicates that a child scans the scene for communicative relevant information (Tenenbaum et al., 2015). In typically developing children, there is a developmental change within the social orientation to faces. This starts with a period of predominant orientation towards eyes, followed by an increased focus on the mouth during language learning, and lastly a decrease of orienting to the mouth with a simultaneous increase in looking to the eyes (Frank et al., 2012). Several studies have found associations between attention to the eyes or mouth of another person and language outcomes, both in typically developing children (e.g., Lewkowicz & Hansen-Tift, 2012; Tenenbaum et al., 2014; Tenenbaum et al., 2015), and children with neurodevelopmental disorders such as autism spectrum disorders (e.g., Habayeb et al., 2021; Stagg et al., 2014; Young et al., 2009). These studies show that these viewing behaviors are not only predictive of concurrent, but also longitudinal language outcome.

When orienting to faces, the direction of the gaze of the other person matters. Young infants already show a sensitivity to deviations in eye gaze direction, with more attention to the eyes of a person when in direct eye contact in contrast to looking away (Symons et al., 1998). This seems to differ between typically developing children and children with neurodevelopmental disorders; however, with typically developing children being more sensitive to direct gaze in contrast to averted gaze, whereas children with ASD for example, do not appear to differentiate between gaze type (Frischen et al., 2007).

Within the SCT population, only a handful of studies assessed social attention abilities in individuals with SCT. For example, in a previous study from this research group which included children from the same population, children with SCT showed reduced attention to the faces and eyes of two people engaged in a social plot and less accurate joint attention skills (Bouw et al., 2021). Studies in XXY adolescents and adults showed diminished attention to eyes while watching affective clips (Van Rijn et al., 2014) or static pictures of facial expressions (Van Rijn, 2015). In addition, adolescents and adults with XXY have a reduced tendency to focus on the eyes when presented with faces (Van Rijn, 2015). It is unknown however, if this diminished spontaneous visual attention towards social aspects in individuals with SCT is already present in early childhood and whether it is related to language outcome.

To understand and interpret individual differences in social orienting, the arousal system also needs to be taken into account. The autonomic nervous system activates and regulates this arousal system during social interactions (Porges, 2001). Arousal is necessary when responding to situational demands; modulation of arousal reflects someone's ability to attend and react in an appropriate manner to environmental demands (Roberts et al., 2008). The ability to modulate arousal differs from person to person. When someone experiences difficulties with modulating arousal levels to the situational demands, this could lead to the development of behavioral and emotional problems (Lydon et al., 2016). If someone experiences too much arousal for example, this can lead to a feeling of being overwhelmed or anxiousness, which subsequently could lead to diminished social participation. Alternatively, if someone experiences too little arousal, this could lead to less motivation to participate, resulting in a diminished focus on others during social encounters (Lydon et al., 2016). One example of a situational demand is eye contact. Eye

contact or direct gaze can affect physiological arousal (Kleinke, 1986). Studies have found greater arousal responses when under direct rather than indirect gaze (for a review see Hietanen, 2018), however these responses have not yet been studied in children with SCT.

Within the SCT population, literature on arousal responses is scarce. One study showed an increased arousal response in adults with XXY when looking at emotional stimuli (Van Rijn et al., 2014). A second study used subjective arousal reports and found increased arousal to emotional events in adults with XXY (Van Rijn et al., 2006). Studies including children with SCT and studies looking at arousal in response to social communication, however, are lacking.

This study has two main aims: First, the social orientation patterns during short nonverbal communicative interactions or 'bids' will be assessed with eye tracking to answer the questions: Which information do children with SCT attend to and what information do they miss? Does gaze direction of the bid (i.e., direct/frontal gaze versus indirect/side gaze) matter? The primary focus will be on the expected difference of attention for social versus nonsocial aspects of the visual scene, and within social aspects specifically on time spent looking at the eyes and mouth of the communicative partner. We also investigated whether the development of viewing patterns towards the eyes and mouth are similar or different in the control and SCT groups and to what degree time spent looking at social aspects of the scene (i.e., the face, eyes, mouth) is associated with language outcomes, both concurrently and one year later. Second, the arousal response during the communicative bids will be assessed to determine how the autonomic nervous system responds to communicative bids, with a focus on similarities or differences in response to a direct or indirect gaze (i.e., the sensitivity to differences in gaze direction). For both aims, the SCT group will be compared to the control group, and the impact of specific SCT karyotype will be assessed as well. Lastly, several additional research questions were addressed, including if time of diagnosis, ascertainment bias, and research site played a role in explaining viewing patterns and arousal responses.

Method

Participants

The present study is part of a larger ongoing project (TRIXY Early Childhood Study) at Leiden University. The TRIXY Early Childhood Study is a longitudinal study that aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. Within the present study, children aged 1-7 years at enrollment were included.

Recruitment took place in the Netherlands, Belgium, and Colorado USA. Children with SCT were recruited with the help of clinical genetic departments, pediatricians, and national support and advocacy groups. Children in the control group were recruited with help of public institutions (e.g., public daycare centers and primary schools) and via the civil registry. Recruitment of the control group took place in the western parts of the Netherlands. Assessments took place at the Trisomy of the X and Y (TRIXY) Expert Center the Netherlands and the eXtraordinarY Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado.

For both the SCT as well as the control group, the following exclusion criteria applied: A history of traumatic brain injury, severely impaired hearing or sight, neurological illness, or colorblindness. Specific for the control group, children with a previous diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association,

2013) were excluded. In addition, as inclusion criterion for both groups, both the child and the (primary) parent/caregiver had to speak Dutch or English. All children had normal or corrected-to-normal vision. Specific for the SCT group, children were included if the trisomy was present in at least 80% of the cells, as confirmed by standard karyotyping. Due to ethical reasons, genetic screening was not performed in the control group. However, based on the prevalence of SCT, the risk of including a child with SCT in the control group was considered minimal and acceptable.

In total 107 children with SCT (33 XXX, 50 XXY, 24 XYY) and 102 controls (58 XX, 44 XY) were included. Ages ranged from 1.00-7.66; years, mean age did not differ between the SCT (M = 3.68, SD = 1.94) and control group (M = 3.61, SD = 1.62; p = .751). Global intellectual functioning was assessed with the Bayley Scales of Infant and Toddler Development (third edition; Bayley, 2006), the Wechsler Preschool and Primary Scale of Intelligence (third edition; Wechsler, 2002) or the Wechsler Nonverbal Scale of Ability (Wechsler & Naglieri, 2006). On average, global intellectual functioning was lower in the SCT (M = 96.58, SD = 17.63) than the control group (M = 105.70, SD = 14.34; p < .001). As a proxy for social economic status (SES), parents were asked to report the highest level of completed education. If two caregivers were present (96.2%), SES was computed as the average of both caregivers. The Hollingshead criteria were used to account for differences in educational systems between countries (Hollingshead, 1975). On average, SES was higher in the SCT group (M = 5.92, SD = .94), than in the control group (M = 5.43, SD = 1.40; p = .003). Children recruited in the USA where White (88.1%), Black or African American (3.4%) or Asian (3.4%) or 'unknown' (5.1%). Information on race/ethnicity in the sample recruited in Western-Europe was not available. Descriptive statistics for age, global intellectual functioning, and SES between the SCT and control group and between the SCT karyotypes can be found in Table 1.

Table 1. Descriptives SCT versus control and SCT karyotypes

	SCT	Control	*p* (SCT vs Control)	XXX	XXY	XYY	SCT comparisons[d]
N	107	102		33	50	24	
Age (years)	3.65 (1.91)	3.61 (1.62)	.865	4.26 (1.74)	3.25 (1.93)	3.80 (2.05)	.062
GIF[a]	96.58 (17.63)	105.70 (14.34)	<.001	94.69 (16.33)	99.48 (17.73)	92.86 (19.00)	.275
SES[b]	5.92 (.94)	5.43 (1.40)	.003	5.91 (1.03)	6.06 (.88)	5.67 (.90)	.239
Ascertainment Bias[c] (A/B/C)				11/12/10	28/15/7	16/3/5	.063
Time of Diagnosis (Prenatal/Postnatal)				20/13	35/15	16/8	.675

Note: scores represent Means (SD)
[a] Data for 6 children with SCT was incomplete (1 XXX, 2 XXY, 3 XYY)
[b] Data for 1 child with SCT was not available
[c] A = Active prospective follow-up; B = Information seeking parents; C = Clinically referred cases
[d] SCT comparisons: XXX versus XXY versus XYY
Abbreviations: GIF = global intellectual functioning / IQ; SES = social economic status

Within the SCT group 71 children received a prenatal diagnosis of SCT as a result of prenatal screening or screening for example due to advanced maternal age. Children that received a postnatal diagnosis (N = 36) received a diagnosis of SCT due to a developmental delay (N = 15), physical and/or growth problems (N = 12) or medical concerns (N = 9). In addition to time of diagnosis, the reason families enrolled in the study was monitored (i.e., ascertainment bias). Three subgroups were identified: 1) 'Active prospective follow-up' (51.4%

of the SCT group), 2) 'Information seeking parents' (28.0% of the SCT group), and 3) 'Clinically referred cases' (20.6%) of the SCT group. Distributions in time of diagnosis and ascertainment bias were similar between the three SCT karyotypes (see Table 1.).

Instruments

Communicative Bids Paradigm

The paradigm consisted of two dynamic video clips of 30 seconds each. In both video clips children were shown a scene of naturalistic caregiver interaction; a female actress smiling and trying to engage using simple universal sounds (e.g., 'hi', 'oh'). The use of language during a communicative bid might be a confounding factor, where children do not necessarily attend to social aspects of a scene naturally, but rather attend to the eyes or mouth of the communicator as a response to hearing language (Brooks & Meltzoff, 2005). For that reason, no speech other than simple sounds were used in the paradigm of this study. Two objects were positioned on the left and right of the actress. In the first video clip, the actress looked directly at the child (frontal gaze direction), whereas in the second video clip the actress was facing sideways – looking towards a point at the right of the child (side gaze direction). Dynamic video clips were used, as the ecological validity is higher for dynamic video clips rather than static pictures. See Figure 1 for a still of the dynamic video clips.

Figure 1. Communicative bids paradigm: Frontal gaze direction (left) and side gaze direction (right)

Eye Tracking: Apparatus

Eye gaze data was collected with a Tobii X2-60 eye tracking device, which records the X and Y coordinates of the position of the eye using a corneal reflection technique (Tobii Technology AB, Danderyd, Sweden). Stimuli were shown on a 15.6-inch laptop with a resolution of 1920x1080 pixels. A sampling frequency of 60 Hz was used.

Eye Tracking: Processing Procedure

Gaze data was processed with Tobii studio version 3.4.8. The Tobii-IV fixation filter was used for defining visual fixations (Olsen, 2012). Areas of interest (AOI) included the total screen, objects, and face, eyes, and mouth of the actress and were drawn with the 'dynamic AOI' tool in Tobii studio. An extended region of 1 cm surrounding the AOI was included to create sufficiently large AOI, as large AOI are more robust to noise (Hessels et al., 2016). There was no overlap between AOI. Both total visit duration and total fixation for the AOI were assessed.

Physiology: Apparatus

Heartrate was used as an indicator for arousal levels. Heartrate data was collected AcqKnowledge (version 5.0.2; BIOPAC Systems Inc.). Recordings were acquired with an Electrocardiogram amplifier (ECG100C) and a BIOPAC data acquisition system (MP150

Windows) at a sampling rate of 1000 Hz. Heart rate was recorded simultaneously with the eye tracking data. The physiological equipment was synchronized with the Tobii software with markers representing the start of the video clips.

Physiology: Processing Procedure

Heartrate data was processed with PhysioData Toolbox v0.5 (Sjak-Shie, 2019). Recorded data was manually inspected by detecting R peaks. With visual identifications, motion artifacts were identified and excluded from the data.

Receptive and Expressive Semantic Language Skills

One-year-olds

In the one-year-old children, semantic language skills were assessed with the Bayley Scales of Infant and Toddler Development – Language scale (Bayley, 2006). This scale consists of separate subtest for receptive and expressive semantic skills. In the receptive subtest, depending on the age of the child, pre-verbal behavior, ability to identify objects and pictures, and understanding of verbal messages was assessed. In the expressive subtest, depending on the age of the child, pre-verbal communication and the ability to name objects and pictures was assessed.

Three-to-seven-year-olds

In children aged 3 years and older, receptive semantic skills were assessed with the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997, 2005). The PPVT assesses the child's listening comprehension to spoken words, where the child must identify the picture (out of 4 pictures) that is orally presented by the researcher. Expressive semantic skills were assessed with the Clinical Evaluation of Language Fundamentals Preschool edition (CELF-P; Wiig et al., 2004, 2012). The CELF-P assesses the child's ability to label people, objects, and actions based on colored images.

Procedure

This study was approved by the Ethical committee of Leiden University Medical Center, the Netherlands, and the Colorado Multiple Institutional Review Board (COMIRB) in Colorado, USA. Written informed consent according to the declaration of Helsinki was obtained after providing a description of the study to the parent(s) of the child.

Assessments took place in either a quiet room at the university or at home. As assessments took place at various sites (the Netherlands, Belgium, Colorado USA), the test set-up and research protocol were identical on all sites. Researchers from the Dutch site were responsible for project and data management (i.e., training and supervision of researchers, processing and scoring of data).

Language assessments were administered in either Dutch or English. All tests were administered according to the standardized procedure as specified in the instrument's manual. Neurocognitive assessments, including assessment of receptive and expressive language took place before the eye tracking and physiology assessments to get the child acquainted with the examiner and testing location. For the eye tracking and physiology assessments, the laptop with the eye tracker was placed on an adaptable table to adjust to the height of the child. The table was placed in a small tent to minimize diversions. The child was seated in a comfortable car seat at approximately 65-centimeter viewing distance. Recording electrodes were placed on the child in the presence of the parent. To familiarize the child with the electrodes, and for the

electrode to properly attach to the skin, the child watched a movie on the laptop for 5-10 minutes before the eye tracking and physiological recording. One electrode was placed 10 cm below the suprasternal notch, a second electrode was placed 10 cm above the bottom of the rib cage on the right side of the child. A ground electrode was included by simultaneously recording electrodermal activity (not included in the current study).

Before the paradigm was shown, a 5-point calibration procedure was conducted. The video clips were shown in a fixed order, all preceded by an attention grabber (i.e., a moving picture of an animal shown on a black background, accompanied by a sound). First, a three-minute resting clip was shown to assess baseline arousal levels. During this time, children looked at fish in an aquarium. Next, the frontal video clip was shown, followed by a 30 second resting clip showing a ball and a slide. Finally, the side video clip was shown. The researcher sat on the left of the child and controlled the Tobii via a remote keyboard. A second researcher controlled the BIOPAC. All physiology equipment was placed outside the sight of the child. Children were instructed to sit quietly and watch the video clips.

Study Design

The TRIXY early childhood study is a longitudinal study with an initial baseline and a one-year follow-up assessment. Within the present study, eye tracking data and arousal assessments during the communicative bids paradigm from the initial assessment were reported for both the SCT and control group. For language assessments, outcomes from the initial assessment as well as the follow-up assessment were reported for the SCT group only. Follow-up language outcomes in combination with valid baseline eye tracking data was available only for a subset of the SCT group ($N = 47$), with follow-up assessments taking place 47-61 weeks after initial assessment ($M = 53$, $SD = 2.64$). The high number of missing data is largely due to the worldwide COVID-19 pandemic, where families were unable to participate and/or assessments had to be postponed (i.e., took place > 18 months after baseline; $N = 26$). Other reasons included invalid eye tracking data ($N = 23$), or families were unable to schedule visits due to family circumstances ($N = 6$).

Statistical Analyses

For the eye tracking data, variables were computed to represent the proportion of time children looked at each AOI. First, for the frontal and side clips separately, the attention to the screen was calculated by dividing each child's total visit duration to the screen by 30 (i.e., the duration of the clip) and multiplied by 100. Next, the percentage of time a child fixated to the objects, face, eyes, and mouth was calculated by dividing the total fixation duration for the AOI by the proportion of time the child attended to the screen and multiplied by 100. Main interests in this study were the total time children attended to the screen, the time children spent looking at social versus nonsocial aspects of the scene, and the time children spent looking at the eyes versus the mouth of the actress.

For the physiological data, the first 30 seconds of the baseline clip were considered as 'baseline arousal level'. Heartrate data that was collected during the social attention bids eye tracking paradigm was summarized in 10 second epochs. Delta scores (Δ) were computed by subtracting the baseline arousal level from the heartrate for each epoch.

Several parametric and non-parametric tests were used. First, to compare SCT versus control children, independent samples t-tests or Repeated Measures MANOVA were used to compare outcomes. If there was unequal variance-covariance (i.e., Box's M $p < .05$), Pillai's

trace was used to interpret the effect. Significant interaction effects were followed by within-group paired samples t-tests. Second, to assess the impact of SCT characteristics, non-parametric Kruksal Wallis tests or MAN(C)OVA were used, depending on sample sizes and comparability of age between groups. Second, to assess the effect of age on outcomes PROCESS moderation analyses were used (Hayes, 2017). The interaction effect between research group and either time spent looking at the eyes or mouth or arousal levels was examined and if applicable followed up with correlations for the SCT and control group separately. Lastly, correlations were calculated between eye tracking outcomes and both concurrent and future language outcomes in children with SCT.

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) Version 25. Level of significance was set at $p \leq .05$. Effect sizes were calculated with partial η^2 and interpreted according to the guidelines by Cohen (1988), with partial η^2 .01 considered as small, partial η^2 .06 as medium, and partial η^2 .14 as large.

Results

Eye Tracking: Communicative Bids - Preliminary Analyses

Data for the eye tracking paradigm was missing for 20 children (9.6%; N_{SCT} = 16), due to technical issues or fatigue of the child. As an indication for the reliability of the data, the total proportion of time children spent looking at the screen (for the frontal and side gaze direction separately) was screened for children who did not contribute sufficient data (30% or 10 seconds). For 15 children (7.2%; N_{SCT} = 10) the data for one or both of the gaze directions was deemed insufficient, and these children were discarded from the analyses. After exclusion of these children, Z-scores were calculated for each of the AOI of interest for the SCT and control group separately. Depending on the analysis, a filter was used to select children with appropriate Z scores between -3 and 3 (i.e., for the Social vs Objects analysis only children with $-3 > Z < 3$ for the AOI face and objects for both the frontal and side gaze direction were included).

Overall, 174 children successfully completed the eye tracking paradigm with reliable data (81 SCT and 93 controls). As an indication of overall attention to the paradigm, attention to the screen collapsed for frontal/side gaze direction was used. On average, children attended to the video 90.4% of the time the videos were displayed. An independent samples t-tests indicated similar attention to the screen between the SCT (89.4%) and control group (91.3%, p = .245).

As there were significant differences in IQ and SES between the SCT and control group, correlations were calculated between these variables and three global eye tracking outcome measures (screen, face, objects collapsed for frontal/side gaze direction). No significant correlations were found (see supplementary materials, Table A.), therefore, IQ and SES were not included in further analyses regarding eye tracking outcomes.

Eye Tracking: Communicative Bids - Attention to Social versus Nonsocial Information

In total, 78 children in the SCT group and 89 children in the control group were included in the social versus nonsocial analysis. The proportion of time children spent looking at social (i.e., the face of the actress) versus nonsocial (i.e., objects on the sides of the actress) aspects of the scene was analyzed for the factor 'gaze direction' (frontal versus side), with research group (SCT versus control) as a between subjects variable. The Repeated Measures MANOVA showed a significant main multivariate effect of research group, Wilks' Lambda = .95, F(2,164)

= 4.46, p = .020, partial η^2 = .05 and a significant main multivariate effect of gaze direction, Wilks' Lambda = .95, $F(2,164)$ = 4.27, p = .016, partial η^2 = .05. The interaction effect of Research group x gaze direction was not significant, Wilks' Lambda = .99, $F(2,164)$ = .80, p = .451, partial η^2 = .01. The significant main effects were further analyzed with univariate tests.

Regarding the main effect of gaze direction (frontal versus side), univariate tests for objects showed that children, regardless of research group, spent proportionally more time looking at the objects in the frontal gaze direction (EMM = 11.52, SE = .73) compared to the side gaze direction (EMM = 9.71, SE = .58), p = .012. Partial η^2 = .04, indicating a small effect. No differences between gaze direction were found for time spent looking at the face, p = .511. Regarding the main effect of research group, results showed that, regardless of gaze direction, children with SCT spent proportionally less time looking at the face (EMM = 47.39, SE = 2.10) than children in the control group (EMM = 55.27, SE = 1.96), p = .007, partial η^2 = .04, indicating a small effect. No significant differences between children with SCT and the control group were found for time spent looking at objects (p = .362).

To evaluate if significant deviations in the SCT group in terms of overall looking time towards the face (irrespective of gaze direction) was impacted by specific SCT karyotype, a nonparametric Kruskal Wallis test was used for a more in-depth analysis within the SCT group. No significant subgroup effects were found (p = .090); indicating that there were no significant differences in attention to faces between the three karyotypes (XXX, XXY, XYY).

Taken together, these results indicate that both children with SCT and controls do not seem to differentiate between gaze direction (frontal/side) when looking at a face, but children in both groups do tend to look more at objects during a direct (frontal) compared to an indirect (side) communicative bid. In addition, compared to controls, children with SCT are less inclined to fixate on the face during a communicative bid, but attend equally towards nonsocial objects. This diminished attention to the face appears to be irrespective of SCT karyotype.

Eye Tracking: Communicative Bids - Eyes versus Mouth

In total, 77 children in the SCT group and 91 children in the control group were included in the eyes versus mouth analysis. The proportion of time children spent looking at the eyes versus the mouth was analyzed for the two gaze directions (frontal versus side), with research group (SCT versus control) as a between subjects variable. The Repeated Measures MANOVA showed a significant main multivariate effect of research group, Pillai's Trace = .04, $F(2,165)$ = 3.79, p = .025, partial η^2 = .04 and a significant main multivariate effect of gaze direction, Pillai's Trace = .10, $F(2,165)$ = 9.12, p < .001, partial η^2 = .10. The interaction effect of Research group x gaze direction was not significant, Pillai's Trace = .01, $F(2,165)$ = .63, p = .537, partial η^2 = .01. The significant main effects were further analyzed with univariate tests.

Regarding the main effect of gaze direction (frontal versus side), univariate tests for attention to the mouth showed that children, regardless of research group, spent proportionally more time looking at the mouth of the actress in the frontal gaze direction (EMM = 16.13, SE = 1.18) compared to the side gaze direction (EMM = 13.13, SE = 1.10), p < .001. Partial η^2 = .07, indicating a moderate effect. There was no effect of gaze direction on time spent looking at the eyes of the actress, p = .110. Regarding the main effect of research group, results showed that, regardless of gaze direction, children with SCT spent proportionally less time looking at the eyes (EMM = 18.40, SE = 1.95) than children in the control group (EMM = 23.94, SE = 1.79), p = .038. Partial η^2 = .03, indicating a small effect. No significant differences between

children with SCT and the control group were found for time spent looking at the mouth of the actress (p = .418).

To evaluate if significant deviations in the SCT group in terms of overall looking time towards the eyes (irrespective of gaze direction) was impacted by specific SCT karyotype, a nonparametric Kruskal Wallis test was used for a more in-depth analysis within the SCT group. No significant differences were found (p = .596); indicating that there were no significant differences in attention to eyes between the three karyotypes (XXX, XYY, XYY).

Taken together, these results indicate that both children with SCT and controls do not differentiate between gaze direction (frontal/side) when looking at eyes, but they do tend to look more at the mouth during a direct compared to an indirect communicative bid. In addition, compared to controls, children with SCT are less inclined to fixate on the eyes during communicative bids, but attend equally to the mouth. This diminished attention to the eyes appears to be irrespective of SCT karyotype.

Eye Tracking: Communicative Bids - Effect of Age

The effect of age on fixation to the eyes and mouth was explored with Process analyses and followed up with correlations. As previous analyses showed no significant research group x gaze direction interactions for the mouth nor the eyes, the frontal and side gaze direction were collapsed as an indication for the overall fixation to the mouth or eyes.

First, for time spent looking at the eyes, age by group interactions were explored with a PROCESS analysis with time spent looking at the eyes as dependent variable, research group as independent variable, and age as moderator. Group effects were not significantly moderated by age, t = .57, p = .570. This indicates that differences between the children with SCT and controls in time spent looking at the eyes were stable across ages. To further examine this relationship, correlations between time spent looking at the eyes and age were calculated for the SCT and control group separately. In both the control and SCT group, there were weak but significant correlations between age and time spent looking at the eyes (controls: r = .21, p = .051; SCT: r = .23, p = .047). In other words, for both children with SCT and controls, children spent more time looking at the eyes when age increased. A visualization of these results can be found in Figure 2.

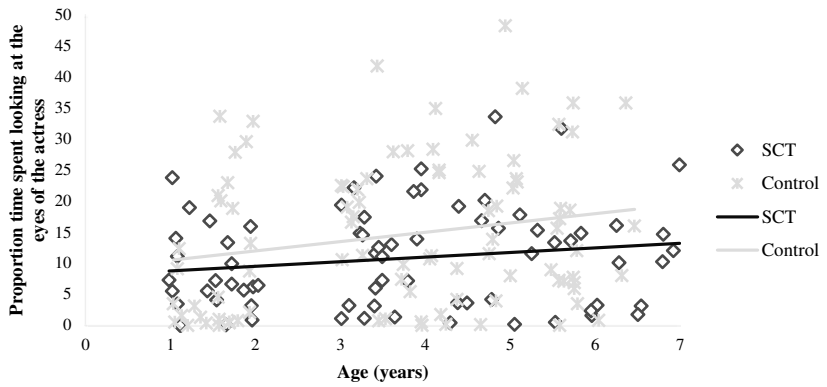

Figure 2. Proportion time attended to the eyes in the SCT and control group at different ages

Second, for time spent looking at the mouth, age by group interactions were explored with a PROCESS analysis with time spent looking at the mouth as dependent variable, research group as independent variable, and age as moderator. Group effects were not significantly moderated by age, t = -.87, p = .384. This indicates that differences between the children with SCT and controls in time spent looking at the mouth were stable across ages. To further examine this relationship, correlations between time spent looking at the mouth and age were calculated for the SCT and control group separately. In the control group, there was a weak but significant relation between age and time spent looking at the mouth, r = -.22, p = .039; when age increased, children in the control group attended less to the mouth. The relationship between age and time spent looking at the mouth in the SCT group however, failed to reach significance, r = -.15, p = .181. This indicates that although time spent looking at the mouth might decrease in the SCT group as well, this decrease was not statistically significant. A visualization of these results can be found in Figure 3.

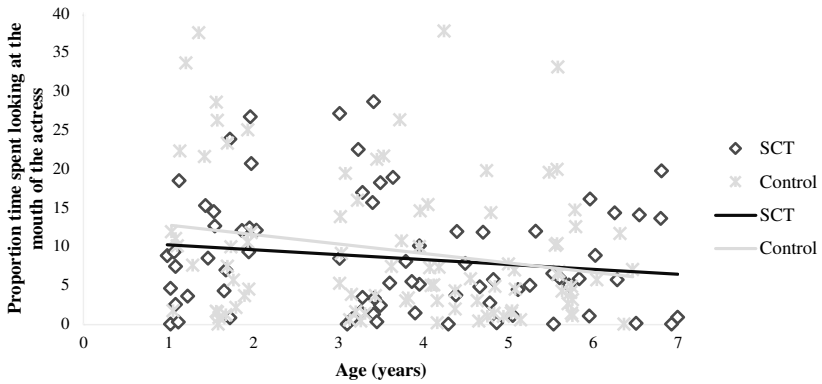

Figure 3. Proportion time attended to the mouth in the SCT and control group at different ages

Attention to the Eyes and Mouth: Correlations with Language Outcomes

Within the SCT group, correlations were calculated between the proportion children spent looking at an AOI (face, eyes, mouth) and both concurrent and future language skills (i.e., at one-year follow-up). To control for age effects, the SCT group was split into three age groups: 1-year-olds, 3-4-year-olds, and 5-7-year-olds. These age groups were comparable in the distribution of karyotypes ($p_{concurrent}$ = .155; p_{future} = .262) and ascertainment bias ($p_{concurrent}$ = .281; p_{future} = .514). Regarding time of diagnosis, there was a difference in the distribution between the age groups when including concurrent language outcomes (p = .011), with more prenatal diagnoses in the younger age group, but there was no difference in the distribution of time of diagnosis between the age groups when including future language outcomes (p = .080).

For the 1-year-olds with SCT, significant correlations were found for looking at the eyes and mouth and both concurrent and future language skills. More specifically, in one-year old children with SCT, children who attended more to the mouth of the actress had significantly better concurrent and future receptive and expressive semantic skills. Simultaneously, time spent looking at the eyes of the actress was significantly negatively correlated with concurrent expressive semantic skills, and future receptive and semantic skills. For the 3–4-year-old children with SCT, no significant relations were found between time spent looking at the eyes

or mouth and concurrent or future language skills. In the children with SCT aged 5-years and older, a trend was found between time spent looking at the mouth and future receptive semantic skills; although not significant, this could indicate that looking at the mouth could be associated with better receptive semantic skills at the follow-up assessment. Correlations per age-group can be found in Table 2.

Table 2. Correlations between current (c) and future (f) language (1-year follow-up) in children with SCT

	1-year-olds $N_c = 22 / N_f = 16$			**3-4-year-olds** $N_c = 28 / N_f = 16$			**5-7-year-olds** $N_c = 22/23 / N_f = 15$		
	Face	Eyes	Mouth	Face	Eyes	Mouth	Face	Eyes	Mouth
Current receptive semantic skills	.19	-.36	.66***	.16	.14	.08	.01	-.15	.18
Current expressive semantic skills	.21	-.45*	.65**	-.12	.05	-.17	.26	.33	.19
Future receptive semantic skills	.09	-.66**	.59**	-.02	.24	-.26	.28	.22	.43[a]
Future expressive semantic skills	.26	-.71**	.74**	-.05	.16	-.02	.19	.22	.03

* $p < .05$; ** $p < .01$; *** $p < .001$ (one-sided)
[a] Trend towards significance, $p = .055$

Arousal Response for Different Gaze Directions: Preliminary Analyses

Children who had missing data or a low reliability on the eye tracking measures were excluded in the arousal analyses as well (16.8%). In addition, children with unreliable physiology data, for example due to a large amount of motion artifacts or malfunctioning hardware, or children who had no (reliable) baseline data were excluded (9.6%). After exclusion of this data, Z-scores were requested for the six 10 second epochs for the SCT and control group separately. A filter was used to select data, only including children with Z scores between -3 and 3 in the analysis.

Overall, 149 children with reliable data were included in the arousal analyses (65 children with SCT and 84 controls). There was no significant difference in average baseline heartrate between the SCT group ($M = 102.27$, $SD = 16.22$) and control group ($M = 101.93$, $SD = 13.92$). There were, however, significant differences between the three SCT karyotypes, with higher baseline HR in the XXY compared to the XXX and XYY group, and the latter not significantly different. To account for individual within-group differences in baseline heartrate, delta (Δ) scores were computed by subtracting the baseline heartrate from the heartrate for each epoch.

First, to evaluate the effectivity of the paradigm in triggering the arousal system, the effect of the frontal and side gaze directions over time was assessed in the control group only. A Repeated Measures ANOVA with gaze direction (frontal versus side) and time (Δ-scores in 3 epochs) revealed a significant interaction effect between gaze direction and time ($p < .001$). Paired samples t-test per epoch (e.g., frontal 1 vs side 1) revealed that the arousal response in the control group differed between the frontal and side gaze direction in the first epoch (10 seconds, $p < .001$), but not in the remaining epochs (p ranging from .332 - .475). This illustrates that children in the control group had a different initial arousal response to the frontal versus side gaze direction; in other words, there was a sensitivity for gaze direction in the first stages of communication. To assess the arousal response in children with SCT in this sensitive time window, only Δ-scores in the first epochs for the frontal and the side gaze direction were included in subsequent analyses.

As there were significant differences in IQ and SES between the SCT and control group, correlations were calculated within the SCT group between these outcomes and Δ-arousal scores for the initial 10 seconds (frontal and side gaze) to assess if arousal levels were dependent on IQ or SES. No significant correlations were found (see supplementary materials, Table A.), therefore, IQ and SES were not included in further analyses regarding physiological outcomes.

Arousal Response for Different Gaze Directions: SCT versus Controls

In total, 65 children in the SCT group and 84 children in the control group were included in the analysis. The Δ-arousal levels within the two gaze directions (frontal versus side) were included as within subjects variable with research group (SCT versus control) as a between subjects variable. The Repeated Measures MANOVA showed a significant research group x gaze direction interaction effect, Wilks' Lambda = .96, $F(1,147) = 5.89$, $p = .016$, partial $\eta^2 = .04$.

The significant interaction effect was further explored with post-hoc paired-samples t-tests. Whereas children in the control group had a different initial response to the frontal versus side gaze direction: A stronger response to the side (ΔHR = -4.94, *SD* = 5.34) compared to the frontal gaze direction (ΔHR = -1.71, *SD* = 6.31), a different pattern was found in the SCT group. In the SCT group, the paired-samples t-test of the did not indicate a difference in arousal response to the gaze direction, $t(64) = 1.09$, $p = .281$, with similar responses in the frontal (ΔHR = -2.50, *SD* = 6.61) and the side gaze direction (ΔHR = -3.26, *SD* = 5.14). A visualization can be found in Figure 4.

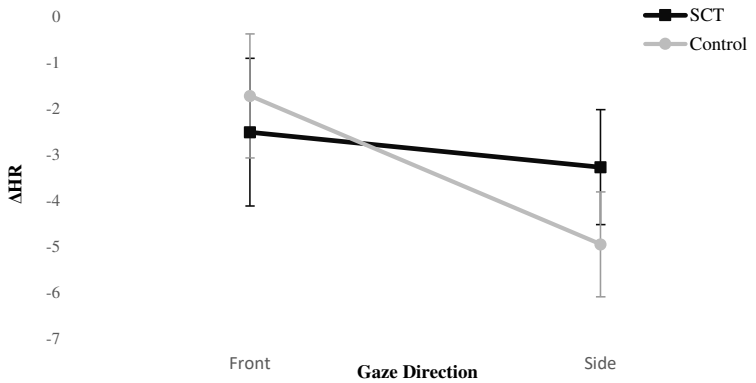

Figure 4. Sensitivity to direction of eye gaze in the SCT and control group

To evaluate if significant deviations in the SCT group in terms of this reduced arousal sensitivity was impacted by specific SCT karyotype, a nonparametric Kruskal Wallis test was used for a more in-depth analysis within the SCT group. A $\Delta HR_{sensitivity}$ score was calculated by subtracting the ΔHR in the side gaze direction from the ΔHR in the frontal gaze direction (first epoch only), where a higher score indicates more sensitivity to gaze direction. With a nonparametric Kruskal Wallis test, gaze direction sensitivity was compared between the three karyotypes (XXX, XXY, XYY). No significant differences were found ($p = .869$) indicating that there are no differences in sensitivity to gaze direction between the three karyotypes.

Collectively, these results indicate that compared to controls, the arousal system of children with SCT appears to be less sensitive to gaze direction. These findings appear to be irrespective of SCT karyotype.

Arousal Response for Different Gaze Directions: Effect of Age

The effect of age on sensitivity to differences in gaze direction was explored with a Process analysis and followed up with correlations. For these analyses $\Delta HR_{sensitivity}$ was used as an indication of gaze direction sensitivity, with higher scores indicating more sensitivity.

A PROCESS analysis with arousal sensitivity as dependent variable, research group as independent variable, and age as moderator showed that group effects were not significantly moderated by age, t = .1.61, p = .110. This indicates that the pattern of sensitivity to gaze direction across ages is not statistically different for children with SCT and controls. To further examine this relationship, correlations between arousal sensitivity and age were calculated for the SCT and control group separately. No significant correlations between age and sensitivity to gaze direction were found in either the control (r = .12, p = .290) or SCT group (r = -.16, p = .215). In other words, for both children with SCT and controls, gaze direction sensitivity (i.e., arousal level) was relatively stable across the age range. A visualization of these results can be found in Figure 5.

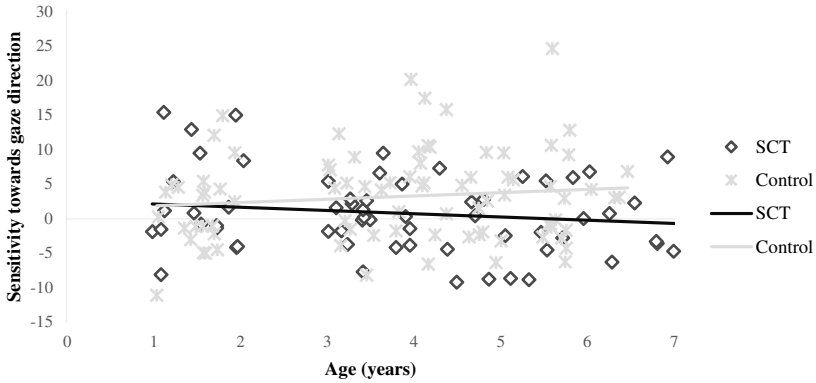

Figure 5. Arousal sensitivity towards gaze direction in the SCT and control group at different ages

Impact of SCT Characteristics on Social Orientation and Arousal Sensitivity

Additional analyses within the SCT group were done to assess to what degree differences between the SCT and control group were impacted by characteristics of the SCT group (i.e., time of diagnosis, ascertainment bias, research site). For the eye tracking outcomes, time spent looking at the face and eyes was explored further (collapsed for gaze direction), for arousal outcomes, sensitivity to gaze direction was explored further. All outcomes can be found in the supplementary materials (Table B.). When comparing SCT subgroups, these subgroups did not differ in distribution of karyotypes, time of diagnosis, and ascertainment bias (when applicable). When comparing subgroups that differed in age, age was included as covariate in the analysis.

Regarding time spent looking at the face, no differences were found between children with a prenatal versus postnatal diagnosis (p = .881), for children who were actively followed-up, information seeking, or clinically referred (i.e., ascertainment bias; p = .821), or for children from the USA versus EU site (p = .262). Regarding time spent looking at the eyes, no differences were found between children with a prenatal versus postnatal diagnosis (p = .248), for children who were actively followed-up, information seeking, or clinically referred (i.e., ascertainment bias; p = .432), or for children from the USA versus EU site (p = .117). Lastly, regarding sensitivity to gaze direction, no differences were found between children with a prenatal versus postnatal diagnosis (p = .512), for children who were actively followed-up, information seeking, or clinically referred (i.e., ascertainment bias; p = .073), or for children from the USA versus EU site (p = .491).

Discussion

This study aimed to increase knowledge of how young children with SCT respond to short periods of communicative interactions (i.e., communicative 'bids'). Overall, this study shows that children with SCT appear to attend less to the face, and specifically the eyes of another person during communicative bids. In very young children (1-year-olds), social orientation was strongly correlated to both concurrent and future language outcomes at one year follow-up. In addition, the arousal system of children with SCT appears to be less sensitive to differences in gaze directions.

This study used a dynamic eye tracking paradigm, with an actress that smiles and uses simple sounds rather than speech to study responses to communicative bids in an ecologically valid way. Previous studies have shown that language and communicative development are among the most affected neurocognitive outcomes in individuals with SCT (e.g., Boada et al., 2009; Urbanus et al., 2019). It is possible that diminished social attention already present very early in life plays a significant role in this. This study shows that young children with SCT orient less to social aspects during communicative interactions (i.e., the face). However, this does not seem to be due to increased attention towards objects. Further exploring this reduced attention to social aspects showed that children with SCT orient less to the eyes of another person, however orientation to the mouth did not differ from controls. This is particularly striking, as attention to the mouth is believed to be adaptive for language learning, and it could be expected that children with SCT, for whom language is a vulnerable domain, would show deviances in looking towards the mouth. Social orientation was modulated by gaze direction in a similar way to the control group; in other words, children with SCT do not appear to differ in sensitivity to the direction of eye gaze while watching a social scene such as a communicative bid. Taken together, it appears that children with SCT experience difficulties orienting towards social aspects of a scene. It is possible that this reduced attention plays a role in picking up social signs, that are important for adequate communicative competence. Sensitivity to these social signs, such as eye tracking, is important as it may lead to a heightened receptive state for upcoming information (Csibra & Gergely, 2009) and to a better understanding of for example another person's mental state (Farroni et al., 2002). In other words, the ability to orient to social aspects of a social scene facilitates neurocognitive development. As some children with SCT appear to have difficulties with attending to social cues, this could play a role in the increased risk for neurocognitive and neurobehavioral difficulties that are reported in this population (e.g., Urbanus et al., 2019; Van Rijn, 2019).

When looking at the arousal system, and more specifically to evaluate if children with SCT are able to adapt to situational demands (i.e., direct versus indirect gaze), we observed a different pattern compared to the control group. In the control group, the level of arousal was dependent on direction of gaze during the communicative bids, in other words, children in the control group modulated their arousal response to the situation. However, this sensitivity to gaze direction, or arousal modulation, was not observed in the SCT group. Based on the results of this study, it can be suggested that the arousal system of children with SCT may respond differently than that of typically developing children. This could imply that children with SCT can depend less on their arousal system as a social 'compass' during social interactions, which could have consequences for how they respond and behave during these interactions. It is important to further explore arousal responses in social situations to gain a better understanding of how the arousal response relates to outcomes in children with SCT.

In addition to the SCT group as a whole, the role of SCT specific characteristics was also explored, including SCT karyotype (XXX, XXY, XYY), time of diagnosis, ascertainment bias, and research site. For none of the study outcomes (i.e., attention to the face, the eyes, and arousal sensitivity), an effect of these SCT characteristics was found. This suggests that the observed vulnerabilities in social orientation and arousal modulation may represent a rather 'stable' vulnerability associated with the genetic variation. It should be noted however, that results represent the *average* group of children with SCT and that there is always variability in outcomes, where some children are vulnerable, whereas other children will not differ from the control group.

Looking at the eyes and mouth of someone during social interactions may be impacted by the age of the child; younger children may focus more on the mouth during language learning, whereas this preferential looking might gradually shift to a preference to looking at the eyes. Also, sensitivity to difference in gaze direction might differ between younger and older children. For these reasons, the effect of age on group differences in looking times and sensitivity in arousal levels were explored further. No interaction effects were found for either time spent looking at the eyes, time spent looking at the mouth, or sensitivity in arousal modulation. Further examining this effect with correlations indicated that for time spent looking at the eyes, both the SCT and control group showed an increase with age. For time spent looking at the mouth the interaction effect did not indicate a different pattern between groups. However, correlations showed a significant decrease for time spent looking at the mouth in the control group, but not the SCT group. Lastly, for sensitivity in arousal modulation, there was no significant relation with age in either the control or SCT group. Taken together these results indicate that, although there might be differences between groups (i.e., children with SCT look less at the eyes) children with SCT do not appear to deviate from the control group more when they get older. This implies a persistent vulnerability across the entire 1–7-year age range, which suggests that it is possible that this vulnerability is anchored in the brain.

Relationships between looking behaviors and language outcomes, both concurrent and one year later were explored as well. Within the youngest age group (1-year-old children), significant correlations were found with both concurrent and future language outcomes. These results are in line with previous studies in typically developing children, and children with neurodevelopmental disorders, such as ASD (e.g., Habayeb et al., 2021; Lewkowicz & Hansen-Tift, 2012; Stagg et al., 2014; Tenenbaum et al., 2014; Tenenbaum et al., 2015; Young et al., 2009). The high correlations found in this age group illustrate that social orientation and language are intertwined at a very young age. It should be noted however, that no causal conclusions can be drawn from this; it remains unclear if more orientation to the mouth leads to better language abilities, or if children with better language abilities are more able to scan for socially relevant aspects, thus if better language abilities lead to more social orientation. With increasing age, typically developing children show a developmental change in orientation to the eyes versus the mouth (Frank et al., 2012). As a result, attention to specific areas of the face may contribute to language learning during specific developmental stages. Our findings fit with the proposition that with increasing age, attention to the mouth becomes less important for language learning, and that at a certain age, children may have passed this point (Tenenbaum et al., 2014).

When taking the results from the eye tracking and arousal together, the results of this study hint at a reduced ability to understanding and/or responding to social communicative

demands in the environment. In other words, children with SCT might have a broader communication deficit. If children with SCT are less able to adapt to situational demands, this might explain why children with SCT also experience difficulties with language and other aspects of communication (Ross et al., 2008; Ross et al., 2009; St John et al., 2019; Urbanus, Swaab, Tartaglia, Boada, et al., 2021; Urbanus, Swaab, Tartaglia, Stumpel, et al., 2021; Zampini et al., 2020; Zampini et al., 2018), and why there are increased reports of social difficulties and social-emotional behavioral problems (Freilinger et al., 2018; Hong & Reiss, 2014; Urbanus et al., 2020; Visootsak & Graham, 2009). This study illustrates that nonverbal communication, that is needed to navigate social communicative interactions, consist of several important aspects, and that children with SCT experience difficulties with at least some of these aspects in areas of social attention and arousal responses.

This study comes with important clinical and scientific implications. Results of this study suggest that the presence of an extra X or Y chromosome may impact systems involved in social communication, not merely language systems. This is in line with neuroimaging studies, that demonstrate the impact of an extra sex chromosome on cortical regions that are part of the 'social brain' (e.g., Raznahan et al., 2016). It is recommended that future studies and clinicians take into account the broader domain of communication, in addition to structural language outcomes in children with SCT. This should be done from a young age, as both orientation difficulties and reduced arousal modulation were found irrespective of age. In addition, as language develops rapidly at a young age, language difficulties are already present in very young children with SCT, and language and social orientation are highly correlated in young children, results from this study point at an important window of opportunity to target social orientation and language in young children with SCT.

A relative strength of this study was that a large international sample of young children with SCT was included. Within this diverse group, no effect of recruitment site, time of diagnosis, or ascertainment bias was found, indicating that the included sample may be an adequate representation of the population of diagnosed children with SCT. It should be noted that although there were significant differences in IQ and SES between the control and SCT group, IQ and SES were not significantly correlated with our main parameters of interest. This in line with previous work (Van Rijn et al., 2018), illustrating that the use of eye tracking is a reliable measure to assess group differences regardless of level of functioning. In addition to strengths, some limitations of this study should also be noted. Although eye tracking allows for an ecological valid way to study looking behaviors, and we used a naturalistic situation, children might respond differently to watching a video as compared to a real life situation. Although we found reduced attention to the eyes in the SCT group while watching a video clip, we cannot conclude that these children also show reduced attention to the eyes in daily interactions. Also, both the effect of age and the effect of SCT karyotype were assessed separately. Due to the sample sizes, we were not able to look at age dependent effects within SCT karyotypes, which is an important direction for future studies. Sample sizes were smaller for our predictions between social orientation and language over time. Largely due to the world-wide COVID pandemic, we were unable to assess language one year after baseline assessment for some children. This resulted in small sample sizes in some of the age groups. Also, within this study, we only looked at the relationship between semantic language outcomes and social orientation, whereas other aspects of language, such as syntax or pragmatic language, might also be related to social orientation, in particular in older children (Çetinçelik et al., 2021). Lastly, results

showed a diminished arousal modulation, and even though overall arousal level is relevant and interesting it does not inform us about type of emotions that are experienced.

To conclude, this study suggests that young children with SCT may have reduced orientation to social cues in response to social communication. In addition, the arousal system of children with SCT may be less sensitive to social cues. As social orienting abilities were related to longitudinal language abilities in the youngest group of children, this stresses the importance of targeting social orientation in early intervention programs.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.) [Text Revision]. American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596

Bayley, N. (2006). *Bayley scales of infant and toddler development - third edition*. Harcourt Assessment

Berglund, A., Viuff, M. H., Skakkebaek, A., Chang, S., Stochholm, K., & Gravholt, C. H. (2019). Changes in the cohort composition of turner syndrome and severe non-diagnosis of Klinefelter, 47,XXX and 47,XYY syndrome: A nationwide cohort study. *Orphanet Journal of Rare Diseases*, *14*(1), Article 16. https://doi.org/10.1186/s13023-018-0976-2

Bishop, D. V. M., Jacobs, P. A., Lachlan, K., Wellesley, D., Barnicoat, A., Boyd, P. A., . . . Scerif, G. (2011). Autism, language and communication in children with sex chromosome trisomies. *Archives of Disease in Childhood*, *96*(10), 954-959. https://doi.org/10.1136/adc.2009.179747

Boada, R., Janusz, J., Hutaff-Lee, C., & Tartaglia, N. (2009). The cognitive phenotype in Klinefelter syndrome: A review of the literature including genetic and hormonal factors. *Developmental Disabilities Research Reviews*, *15*(4), 284-294. https://doi.org/10.1002/ddrr.83

Bouw, N., Swaab, H., Tartaglia, N., & Van Rijn, S. (2021). The impact of sex chromosome trisomies (XXX, XXY, XYY) on early social cognition: Social orienting, joint attention and theory of mind. *Archives of Clinical Neuropsychology*, Article acab042. https://doi.org/10.1093/arclin/acab042

Brooks, R., & Meltzoff, A. N. (2005). The development of gaze following and its relation to language. *Developmental Science*, *8*(6), 535-543. https://doi.org/10.1111/j.1467-7687.2005.00445.x

Çetinçelik, M., Rowland, C. F., & Snijders, T. M. (2021). Do the eyes have it? A systematic review on the role of eye gaze in infant language development. *Frontiers in Psychology*, *11*, Article 589096. https://doi.org/10.3389/fpsyg.2020.589096

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Lawrence Erlbaum Associates.

Csibra, G., & Gergely, G. (2009). Natural pedagogy. *Trends in Cognitive Sciences*, *13*(4), 148-153. https://doi.org/10.1016/j.tics.2009.01.005

Dawson, G., Toth, K., Abbott, R., Osterling, J., Munson, J., Estes, A., & Liaw, J. (2004). Early social attention impairments in autism: Social orienting, joint attention, and attention to distress. *Developmental Psychology*, *40*(2), 271-283. https://doi.org/10.1037/0012-1649.40.2.271

Dunn, L. M., & Dunn, L. M. (1997). *Peabody Picture Vocabulary Test - Third Edition Manual*. American Guidance Service

Dunn, L. M., & Dunn, L. M. (2005). *Peabody Picture Vocabulary Test-III-NL. Handleiding* (L. Schlichting, Trans.). Harcourt Test Publishers.

Farroni, T., Csibra, G., Simion, f., & Johnson, M. H. (2002). Eye contact detection in humans from birth. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(14), 9602-9605. https://doi.org/10.1073/pnas.152159999

Frank, M. C., Vul, E., & Saxe, R. (2012). Measuring the development of social attention using free-viewing. *Infancy*, *17*(4), 355-375. https://doi.org/10.1111/j.1532-7078.2011.00086.x

Frazier Norbury, C., Brock, J., Cragg, L., Einav, S., Griffiths, H., & Nation, K. (2009). Eye-movement patterns are associated with communicative competence in autistic spectrum disorders. *The Journal of Child Psychology and Psychiatry*, *50*(7), 834-842. https://doi.org/10.1111/j.1469-7610.2009.02073.x

Freilinger, P., Kliegel, D., Hanig, S., Oehl-Jaschkowitz, B., Henn, W., & Meyer, J. (2018). Behavioral and psychological features in girls and women with triple-X syndrome. *American Journal of Medical Genetics Part A*, *176*(11), 2284-2291. https://doi.org/10.1002/ajmg.a.40477

Frischen, A., Bayliss, A. P., & Tipper, S. P. (2007). Gaze cueing of attention: Visual attention, social cognition, and individual differences. *Psychological Bulletin*, *133*(4), 694-724. https://doi.org/10.1037/0033-2909.133.4.694

Groth, K. A., Skakkebaek, A., Høst, C., Gravholt, C. H., & Bojesen, A. (2013). Klinefelter syndrome - a clinical update. *The Journal of Clinical Endocrinology & Metabolism*, *98*(1), 20-30. https://doi.org/10.1210/jc.2012-2382

Guillon, Q., Hadjikhani, N., Baduel, S., & Roge, B. (2014). Visual social attention in autism spectrum disorder: Insights from eye tracking studies. *Neuroscience and Biobehavioral Reviews*, *42*, 279-297. https://doi.org/10.1016/j.neubiorev.2014.03.013

Habayeb, S., Tsang, T., Saulnier, C., Klaiman, C., Jones, W., Klin, A., & Edwards, L. A. (2021). Visual traces of language acquisition in toddlers with autism spectrum disorder during the second year of life. *Journal of Autism and Developmental Disorders*, *51*(7), 2519-2530. https://doi.org/10.1007/s10803-020-04730-x

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach* (Second edition ed.). Guilford Press.

Hessels, R. S., Kemner, C., van den Boomen, C., & Hooge, I. T. (2016). The area-of-interest problem in eyetracking research: A noise-robust solution for face and sparse stimuli. *Behavior Research Methods*, *48*(4), 1694-1712. https://doi.org/10.3758/s13428-015-0676-y

Hietanen, J. K. (2018). Affective eye contact: An integrative review. *Frontiers in Psychology*, *9*, Article 1587. https://doi.org/10.3389/fpsyg.2018.01587

Hillairet de Boisferon, A., Tift, A. H., Minar, N. J., & Lewkowicz, D. J. (2018). The redeployment of attention to the mouth of a talking face during the second year of life. *Journal of Experimental Child Psychology*, *172*, 189-200. https://doi.org/10.1016/j.jecp.2018.03.009

Hollingshead, A. B. (1975). *Four factor index of social status* [Unpublished Manuscript]. Yale University, New Haven, CT.

Hong, D. S., & Reiss, A. L. (2014). Cognitive and neurological aspects of sex chromosome aneuploidies. *The Lancet Neurology*, *13*(3), 306-318. https://doi.org/10.1016/s1474-4422(13)70302-8

Kleinke, C. L. (1986). Gaze and eye contact: A research review. *Psychological Bulletin*, *100*(1), 78-100. https://doi.org/10.1037/0033-2909.100.1.78

Lewkowicz, D. J., & Hansen-Tift, A. M. (2012). Infants deploy selective attention to the mouth of a talking face when learning speech. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(5), 1431-1436. https://doi.org/10.1073/pnas.1114783109

Lydon, S., Healy, O., Reed, P., Mulhern, T., Hughes, B. M., & Goodwin, M. S. (2016). A systematic review of physiological reactivity to stimuli in autism. *Developmental Neurorehabilitation*, *19*(6), 335-355. https://doi.org/10.3109/17518423.2014.971975

Mundy, P., & Neal, R. (2000). Neural plasticity, joint attention, and a transactional social-orienting model of autism. In L. Gidden (Ed.), *International Review of Research in Mental Retardation* (Vol. 20, pp. 139-168). Academic Press. https://doi.org/10.1016/s0074-7750(00)80009-9

Nation, K., & Penny, S. (2008). Sensitivity to eye gaze in autism: Is it normal? Is it automatic? Is it social? *Development and Psychopathology*, *20*(1), 79-97. https://doi.org/10.1017/S0954579408000047

Olsen, A. (2012). *The Tobii I-VT Fixation Filter - Algorithm Description*. Tobii Technology.

Porges, S. W. (2001). The poluvagal theory: Phylogenetic substrates of a social nervous system. *International Journal of Psychophysiology*, *42*, 123-146. https://doi.org/10.1016/s0167-8760(01)00162-3

Raznahan, A., Lee, N. R., Greenstein, D., Wallace, G. L., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2016). Globally divergent but locally convergent X- and Y-chromosome influences on cortical development. *Cerebral Cortex*, *26*, 70-79. https://doi.org/10.1093/cercor/bhu174

Roberts, J., Mazzocco, M. M., Murphy, M. M., & Hoehn-Saric, R. (2008). Arousal modulation in females with fragile X or Turner syndrome. *Journal of Autism and Developmental Disorders*, *38*(1), 20-27. https://doi.org/10.1007/s10803-007-0356-6

Ross, J. L., Roeltgen, D. P., Stefanatos, G., Benecke, R., Zeger, M. P. D., Kushner, H., . . . Zinn, A. R. (2008). Cognitive and motor development during childhood in boys with Klinefelter syndrome. *American Journal of Medical Genetics Part A*, *146A*(6), 708-719. https://doi.org/10.1002/ajmg.a.32232

Ross, J. L., Zeger, M. P., Kushner, H., Zinn, A. R., & Roeltgen, D. P. (2009). An extra X or Y chromosome: contrasting the cognitive and motor phenotypes in childhood in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Developmental Disabilities Research Reviews*, *15*(4), 309-317. https://doi.org/10.1002/ddrr.85

Sigman, M. D., Kasari, C., Kwon, J.-H., & Yirmiya, N. (1992). Responses to the negative emotions of others by autistic, mentally retarded, and normal children. *Child Development*, *63*(4), 796-807. https://doi.org/10.1111/j.1467-8624.1992.tb01662.x

Sjak-Shie, E. E. (2019). *PhysioData Toolbox (Version 0.5)*. In [Computer Software]. Retrieved from https://PhysioDataToolbox.leidenuniv.nl

St John, M., Ponchard, C., van Reyk, O., Mei, C., Pigdon, L., Amor, D. J., & Morgan, A. T. (2019). Speech and language in children with Klinefelter syndrome. *Journal of Communication Disorders*, *78*, 84-96. https://doi.org/10.1016/j.jcomdis.2019.02.003

Stagg, S. D., Linnell, K. J., & Heaton, P. (2014). Investigating eye movement patterns, language, and social ability in children with autism spectrum disorder. *Development and Psychopathology*, *26*(2), 529-537. https://doi.org/10.1017/S0954579414000108

Symons, L. A., Hains, S. M. J., & Muir, D. W. (1998). Look at me: Five-month-old infants' sensitivity to very small deviations in eye-gaze during social interactions. *Infant Behavior and Development*, *21*(3), 531-536. https://doi.org/10.1016/S0163-6383(98)90026-1

Tenenbaum, E. J., Amso, D., Abar, B., & Sheinkopf, S. J. (2014). Attention and word learning in autistic, language delayed and typically developing children. *Frontiers in Psychology*, *5*, Article 490. https://doi.org/10.3389/fpsyg.2014.00490

Tenenbaum, E. J., Sobel, D. M., Sheinkopf, S. J., Shah, R. J., Malle, B. F., & Morgan, J. L. (2015). Attention to the mouth and gaze following in infancy predict language development. *Journal of Child Language*, *42*(6), 1173-1190. https://doi.org/10.1017/S0305000914000725

Urbanus, E., Swaab, H., Tartaglia, N., Boada, R., & Van Rijn, S. (2021). A cross-sectional study of early language abilities in children with sex chromosome trisomy (XXY, XXX, XYY) aged 1-6 years. *Child Neuropsychology*, *28*(2), 171-196. https://doi/org/10.1080/09297049.2021.1960959

Urbanus, E., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2020). The behavioral profile of children aged 1-5 years with sex chromosome trisomy (47,XXX, 47,XXY, 47,XYY). *American Journal of Medical Genetics part C*, *184C*, 444-455. https://doi.org/10.1002/ajmg.c.31788

Urbanus, E., Swaab, H., Tartaglia, N., Stumpel, C., & Van Rijn, S. (2021). Structural and pragmatic language in young children with sex chromosome trisomy (XXX, XXY, XYY): Predictive value for neurobehavioral problems one year later. *submitted*.

Urbanus, E., Van Rijn, S., & Swaab, H. (2019). A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention. *Clinical Genetics*, *97*(1), 156-167. https://doi.org/10.1111/cge.13586

Van Rijn, S. (2015). Social attention in 47,XXY (Klinefelter syndrome): Visual scanning of facial expressions using eyetracking. *Journal of the International Neuropsychological Society*, *21*(5), 364-372. https://doi.org/10.1017/S1355617715000302

Van Rijn, S. (2019). A review of neurocognitive functioning and risk for psychopathology in sex chromosome trisomy (47,XXY, 47,XXX, 47,XYY). *Current Opinion in Psychiatry*, *32*(2), 79-84. https://doi.org/10.1097/yco.0000000000000471

Van Rijn, S., Barendse, M., van Goozen, S., & Swaab, H. (2014). Social attention, affective arousal and empathy in men with Klinefelter syndrome (47,XXY): Evidence from eyetracking and skin conductance. *PLoS One*, *9*(1), Article e84721. https://doi.org/10.1371/journal.pone.0084721

Van Rijn, S., Swaab, H., Aleman, A., & Kahn, R. S. (2006). X Chromosomal effects on social cognitive processing and emotion regulation: A study with Klinefelter men (47,XXY). *Schizophrenia Research*, *84*(2-3), 194-203. https://doi.org/10.1016/j.schres.2006.02.020

Van Rijn, S., Urbanus, E., & Swaab, H. (2018). Eyetracking measures of social attention in young children: How gaze patterns translate to real-life social behaviors. *Social Development*, *28*, 564-580. https://doi.org/10.1111/sode.12350

Visootsak, J., & Graham, J. M., Jr. (2009). Social function in multiple X and Y chromosome disorders: XXY, XYY, XXYY, XXXY. *Developmental Disabilities Research Reviews*, *15*(4), 328-332. https://doi.org/10.1002/ddrr.76

Wechsler, D. (2002). *WPPSI-III: Technical and interpretative manual* The Psychological Corporation

Wechsler, D., & Naglieri, J. A. (2006). *Wechsler Nonverbal Scale of Ability*. Harcourt Assessment

Wiig, E. H., Secord, W. A., & Semel, E. (2004). *Clinical Evaluation of Language Fundamentals - Preschool (2nd ed.)*. Harcourt Assessment

Wiig, E. H., Secord, W. A., & Semel, E. (2012). *Clinical Evaluation of Language Fundamentals Preschool (CELF-2NL) - Nederlandse Versie* (J. de Jong, Trans.). Pearson Assessment and Information.

Young, G. S., Merin, N., Rogers, S. J., & Ozonoff, S. (2009). Gaze behavior and affect at 6 months: Predicting clinical outcomes and language development in typically developing infants and infants at risk for autism. *Developmental Science*, *12*(5), 798-814. https://doi.org/10.1111/j.1467-7687.2009.00833.x

Zampini, L., Burla, T., Silibello, G., Capelli, E., Dall'Ara, F., Rigamonti, C., . . . Vizziello, P. G. (2020). Preverbal skills in 8-month-old children with sex chromosome trisomies. *First Language*, *41*(2), 200-217. https://doi.org/10.1177/0142723720962944

Zampini, L., Burla, T., Silibello, G., Dall'Ara, F., Rigamonti, C., Lalatta, F., & Vizziello, P. (2017). Early communicative skills of children with Klinefelter syndrome. *Clinical Linguistics & Phonetics*, *32*(7), 577-586. https://doi.org/10.1080/02699206.2017.1384853

Zampini, L., Draghi, L., Silibello, G., Dall'Ara, F., Rigamonti, C., Suttora, C., . . . Vizziello, P. (2018). Vocal and gestural productions of 24-month-old children with sex chromosome trisomies. *International Journal of Language & Communication Disorders*, *53*(1), 171-181. https://doi.org/10.1111/1460-6984.12334

Supplementary Materials

Correlations between Parameters and Impact of SCT Characteristics on Outcomes

Table A. Correlations between IQ, SES, and global eye tracking parameters in the SCT group

	Screen	Face	Objects	ΔHR Direct Gaze (frontal)	ΔHR Indirect Gaze (side)
IQ	.09	-.19	.10	.11	.06
SES	-.12	-.08	.00	.04	.19

Table B. Impact of SCT characteristics on eye tracking outcomes: Time of diagnosis, ascertainment bias, and research site

		Time of Diagnosis[a]			Ascertainment Bias[b]				Research Site[a]		
		Prenatal	**Postnatal**	***p***	**A**	**B**	**C**	***p***	**USA**	**NL/BE**	***p***
Eye Tracking	*N*	48	26		33	23	18		38	36	
	Face	28.76	28.35	.881	28.38	29.68	27.68	.821	27.14	30.02	.262
		(1.57)	(2.18)		(11.02)	(11.10)	(8.76)		(1.79)	(1.74)	
	Eyes	10.04	12.53	.248	9.58	12.49	11.37	.432	9.32	12.44	.117
		(1.21)	(1.68)		(8.59)	(8.78)	(7.44)		(1.38)	(1.34)	
Arousal	*N*	39	26		31	21	13		34	31	
	Sensitivity	.341	1.39	.512	1.38	-1.41	2.80	.073	1.24	.23	.491
		(.95)	(1.19)		(6.08)	(4.89)	(4.87)		(.99)	(1.04)	

[a] Comparisons with (M)ANCOVA Scores represented Estimated Marginal Means (SE), corrected for age
[b] Comparisons with (M)ANOVA. Scores represent Means (SD). Ascertainment bias: A = Active prospective follow-up; B = Information seeking parents; C = Clinically referred cases

Chapter 7

Eye tracking measures of social attention in young children: How gaze patterns translate to real-life social behaviors

Published as:

Van Rijn, S., Urbanus, E., & Swaab, H. (2018). Eyetracking measures of social attention in young children: How gaze patterns translate to real-life social behaviors. *Social Development, 28*(3), 564-580. https://doi.org/10.1111/sode.12350

Abstract

The aim of this study was to evaluate to what degree eye tracking paradigms of social attention, in combination with synchronous measurements of affective arousal, were associated with real-life social behavior of children aged 3–7 years. Several eye tracking paradigms were used, involving social interactions, single/multiple faces, and emotional faces. Arousal was measured using electrocardiography. Real-life social behavior was measured using structured behavior observations, parent questionnaires, and developmental interviews. Time spent looking at social stimuli was significantly associated with real-life social behaviors, and independent of age, IQ, or gender. Paradigms involving social interactions and looking time to the eyes showed the most consistent relations with social behaviors. Stronger affective arousal responses were associated with shorter looking times toward eyes, which in turn were associated with less social awareness in real life. Eye tracking and arousal measures allow for sensitive and objective assessment of social abilities that have great relevance for real-life social behaviors, with the potential to use in a broad and diverse population. These measures may help gain insight into the underpinnings of social behavior and may serve as a valuable marker or outcome measure in understanding, monitoring, and stimulating social-emotional development early in life.

Introduction

From a young age, children typically have a preference for social stimuli, such as faces, eyes, and body motions (Chita-Tegmark, 2016). This social attention, which can be described as "coordinating attention during interaction with others", as "motivation to engage with others", or as "attention in the context of social information input" (Salley & Colombo, 2016, p. 689), is fundamental to social development. Early impairments in social attention can deprive a child of social information input, which in turn could disrupt brain and behavioral development (Mundy & Neal, 2000).

Because of the relevance of social attention for social development of children, it has been studied extensively, both in typically developing children and in children with aberrant social development, for example those with Autism Spectrum Disorder (ASD) or social anxiety. This interest not only includes the identification of individual differences in children's social attention, but also the evaluation of how early training or intervention may impact social attention development, which calls for methods to assess social attention in a way that reflects real life social behaviors.

In order to be able to provide in this, it is important that instruments assessing social attention in young children meet several criteria: (a) instruments should be sensitive, in order to pick up small individual differences, (b) instruments should be able to capture social attention independent of IQ and verbal instructions/responses, in order to be able to compare social attention across groups of children who vary in level of intellectual functioning, and (c) instruments should preferably have high ecological validity, in order to extrapolate findings to real life social abilities. Instruments that meet these criteria have the potential to discriminate between children with different social abilities and different developmental trajectories.

Traditionally, behavioral observations or video recordings have been used to measure social attention in young children (Dawson et al., 2004). These experimental designs have led to important insights, such as that social attention is important to acquire communicative competence (Dawson et al., 2004). Interestingly, with advancing technology, it has now become possible to measure social attention with the help of eye-movement recording techniques (Guillon et al., 2014). Eye-movements can be recorded while individuals are presented with pictures or dynamic clips of complex and naturalistic scenes (Ames & Fletcher-Watson, 2010). Such eye tracking paradigms can help capture an individual's perception of the world; what individuals attend to, and which information they may miss (Falck-Ytter et al., 2013). Studies have shown that eye tracking is suitable to assess developmental changes in different aspects of social attention in young children (for example Frank et al., 2012). A range of eye tracking studies have revealed that from infancy children prefer faces and face-like stimuli over non-social stimuli (for a review see Reynolds & Roth, 2018). Attention to social cues, as measured with eye tracking, is related strongly to the ability to learn from social signals, with an age-related increase in social attention within the first year of life (Frank et al., 2014). With the availability of eye tracking techniques and opportunities to study early social development, it has become increasingly important to address how eye tracking of social attention may fulfill the need for sensitive and objective techniques that reflect real life social behaviors.

So far, a range of studies have used eye tracking to show that children with compromised social behavioral development also show abnormal looking behavior (Chita-Tegmark, 2016), suggesting that eye tracking can be used to pick up global group differences in social outcomes. So far only a handful of studies have focused on the relationship between eye tracking and real-life social behaviors. Most of these studies focused on children with atypical social development, with the majority relying on interviews or questionnaires from the parents' point of view as a measure of social behavior, rather than also relying on systematic observations of children's social behaviors. These studies showed that children and adolescents with ASD who fixate less on the eyes of a person when watching a video clip are characterized by more social impairments on several questionnaires and interviews, including the autism diagnostic interview, autism diagnostic observation schedule, vineland adaptive behavior scales, and social responsiveness questionnaire (Falck-Ytter et al., 2010; Jones et al., 2008; Klin et al., 2002; Speer et al., 2007). However, a relation between looking times towards eyes and social competence is not found consistently, and possible explanations that have been given for this discrepancy in findings include participant characteristics and type of stimuli used. For example, (Speer et al., 2007) concluded that differences in the face processing of individuals with ASD only became apparent when the stimuli were realistic and social in nature, which stresses the importance of using stimuli with high ecological validity.

In understanding and interpreting individual differences in social attention in children, it is important also to take into account affective arousal responses, as expressed in autonomic nervous system parameters such as heart rate. Arousal represents one of the dimensions of emotional responsiveness and is considered crucial in order to be able to resonate emotionally with others in social context (Kreibig, 2010). Social stimuli, in particular direct eye-gaze, may impact an individual's affective arousal system (Helminen et al., 2011), which in turn may impact social attention and social behavior; someone who experiences too much arousal, can experience personal distress and may be too overwhelmed to participate adaptively in social encounters. Such increased arousal may for example be downregulated by looking away from the eyes of others (Chen & Clarke, 2017). Alternatively, someone who experiences too little arousal, may not feel motivated (i.e., is understimulated) to focus on others during social encounters (Lydon et al., 2016). There are a few studies that have used physiological arousal measures in combination with eye tracking images in children. However, these studies all were focused on children with atypical social development such as ASD (for example Louwerse et al., 2013; Nuske et al., 2014; Stagg et al., 2013; Zantinge et al., 2017) or social anxiety (Price et al., 2013). Nonetheless, these studies have shown that looking at arousal responses may be helpful in understanding individual differences in social attention (i.e., accompanied by hypo-arousal versus hyper-arousal), and related social behavior.

Taken together, with technological advances that allow for eye tracking assessment of social attention, combined with synchronous measurement of psychophysiological responses (heart rate), there is a need to assess how such experimental paradigms relate to real-life social behaviors. This study will aim to contribute to this gap in research. The key aim of the study was to assess to what degree eye tracking measures of social attention are associated with real life social outcomes. In answering this question this study not only captured real life social

behaviors through parental interpretation as many behavioral questionnaires do, but also used systematic behavior observations of specific social behaviors of children.

In addition, there were several additional exploratory research questions. First, what type of eye tracking stimuli are most strongly related to real-life social behavior? Other studies have shown that dynamic stimuli (particularly those showing social interactions) are more sensitive than static images in detecting individual differences in social cognition (Chevallier et al., 2015; Risko et al., 2012). Also, as scene complexity increases, for example by adding action or social content to a scene, the preference for looking at the eyes is even stronger (Birmingham et al., 2008). Therefore, we selected several dynamic stimuli, including single faces, single faces with emotional expressions, multiple faces, and faces of multiple persons interacting with each other. Second, to what degree are these eye tracking measures of social cognition (in)dependent of IQ and verbal abilities? The answer to this question is relevant considering the opportunities to use eye tracking of social attention in lower functioning children, and to compare social attention across groups that differ in level of functioning. And third, is social attention as measured by eye tracking related to affective arousal triggered by the social stimuli? Including affective arousal measures (such as heart rate) in eye tracking paradigms could help in interpreting eye tracking data in terms of underlying mechanisms driving social attention.

Method

Participants

In total, 32 children (16 boys and 16 girls) participated in the study. Average age was 4;7 years (SD 1;1), ranging from 3;0 years to 6;8 years. All children spoke Dutch as their primary language. The children were recruited at day-care centers and kindergarten schools. Exclusion criteria were intellectual disability (<70 IQ points), known brain trauma, or a neurological disorder. In addition, all children were screened for psychopathology and autism symptoms: none scored in the clinical range (> 95th percentile) on the child behavior checklist (CBCL; Achenbach, 1991) or the social responsiveness scale (SRS; Constantino et al., 2003).

Instruments

Social Behavior

Parent Questionnaire for Social Behavior: Social Responsiveness Scale

The social responsiveness scale (SRS; Constantino et al., 2003) is most often used to quantify social behaviors associated with ASD, which are normally distributed in the general population. The SRS relies on parental report, has five subscales, and yields scores for each of the subscales and one total score. In this study, the total score was used to exclude children who scored in the severe range (T-scores of 76 or higher). In addition, the social communication, social cognition, social motivation and social awareness subscale scores (but not the autistic behaviors subscale) were used to quantify social skills. The SRS has strong internal consistency (α = .95; Constantino & Gruber, 2012), and extensive proof of validity (Bruni, 2014).

Parent Interview for Social Behavior: Vineland Adaptive Behavior Scales

Socialization skills of the child were measured with the vineland adaptive behavior scales second edition (VABS-II; Sparrow et al., 2005). The VABS is a widely used parent interview

that measures the child's level of adaptive functioning in several domains. Studies have shown high construct validity and good reliability in children and adolescents with varying levels of functioning (De Bildt et al., 2005). For this study the total score for the Socialization domain was used. Items on the VABS are scored on a five-point scale (0 = child does not perform behavior [independently]; 1 = child rarely performs behavior independently; 2 = child sometimes performs behavior independently; 3 = child often performs behavior independently; 4 = child always performs behavior independently). These scores provide sum and age-equivalent scores, and a standard score for the domain.

Structured Observations of Social Behavior: Early Social Communication Scales

The Early Social Communication Scales (ESCS; Seibert et al., 1982) is a videotaped, structured, interactive play task designed to assess social and communication skills that are usually acquired in the first 30 months of life. Although the ESCS is typically used in very young children, there are also studies with the ESCS involving children up to six years (McEvoy et al., 1993; Mundy et al., 1990).

In the ESCS the child is seated at a table across from a familiar examiner. The examiner presents a sequence of wind-up and hand-operated toys, which are used to elicit social interaction, joint attention, and/or behavioral requests. The examiner also tries to attract the child's attention, by pointing and gazing at posters (set up behind the child) while calling the child's name, making gestural and verbal requests ("Give it to me"), and presenting the child with turn- taking opportunities. The 20 minutes play session is videotaped, with full face view of the child and profile view of the experimenter.

Three distinct social communicative functions are scored based on the videotaped session: initiating social interaction, initiating joint attention, and initiating behavioral requests. The joint attention subscale was used as a measure of the number of times a child made social contact with the examiner to share attention on a third object. These behaviors included spontaneously showing a toy to the examiner, pointing at objects within reach, or looking at the examiner to direct attention to a toy. Social interaction behaviors included the ability to maintain a simple social interaction such as turn-taking or sharing objects involving a simple social scheme. The behavioral requests scale assessed the child's ability to respond to requests by the examiner and the child's ability to direct another person's behavior in order to obtain a desired object or event. Following procedures described by Mundy et al. (1990), the frequencies of behaviors occurring under each of the three social communicative functions were scored by independent raters (who were not involved in the assessment), based on videotape recordings. Interrater reliability was measured based on a subsample of 24 participants and showed an intraclass correlation coefficient (ICC) of .78 (for the three ESCS scales collapsed), which is considered excellent reliability (Cicchetti & Sparrow, 1981).

Intellectual Ability

The intellectual level of the child was assessed with subtests of the Dutch Wechsler Preschool and Primary Scale of Intelligence (Third edition; WPPSI-III; Wechsler, 2002). Two short forms were used: One for three-year-old participants, and one for participants four-years and older (Campbell, 1998). For an overview of the subtests, see Table 1. Performance on the subtests yielded three scores: Verbal intelligence (VIQ), performance intelligence (PIQ), and an

estimation of the full-scale intelligence (FSIQ). Reliability for the estimated FSIQ is sufficient (α = .88-.94; Campbell, 1998), in addition to high proof of validity (Wechsler, 2002).

Table 1. Subtests of Wechsler preschool and primary scale of intelligence (WPPSI) used for different age groups

	3-year-olds	4-7-year-olds
FSIQ	Receptive vocabulary Information Block design Object assembly	Vocabulary Information Block design Matrix reasoning Picture completion Word reasoning
VIQ	Receptive vocabulary Information	Vocabulary Information Word Reasoning
PIQ	Block design Object assembly	Block design Matrix reasoning Picture completion

Eye Tracking Equipment and Procedures

Gaze data within specific areas of interest (AOIs) were collected using the Tobii X2-60 eye tracker (Tobii Technology AB, Danderyd, Sweden), which records the X and Y coordinates of the child's eye position at 60 Hz by using corneal reflection techniques. The eye tracker was placed on a table adapted to the height of the seat, and the child was seated in a comfortable chair at about a 65-cm viewing distance. Before starting the eye tracking, the Tobii Studio infant calibration procedure (including nine calibration points) was conducted. Then, children were instructed that they would watch some movie clips and pictures on the computer. The session started with an attention grabber (e.g., a moving picture of a cat, shown on a black background and accompanied by a sound) to direct the child's attention to the screen. Then, several eye tracking paradigms were presented in fixed order (Single/Multiple faces, Social interactions, Emotional faces), during which gaze data were collected. Tobii Studio automatically includes only valid data (and excludes missing data) for calculating visit duration (representing the time eyes were on the screen) and fixation duration (total time eyes fixated within an AOI). Gaze data were processed using the Tobii I-VT fixation filter in Tobii Studio (Version 3.2.1). With the "Dynamic AOI" tool, screen AOIs were drawn. The AOIs were drawn with a one-centimeter margin. A "relative" total fixation duration was calculated by taking the total fixation duration within the AOI, divided by the duration of the clip, multiplied by 100, reflecting the percentage of time children were attending to an AOI. In order to evaluate the degree of missing (i.e., nonvalid) eye tracking data, we calculated the total visit duration toward the whole screen, divided by the duration of the clip, multiplied by 100, reflecting the percentage of valid data collected during each of the eye tracking tests.

Eye Tracking Stimuli

Eye Tracking of Social Interactions

For this paradigm a 30 second video clip was used, displaying a social plot with two actors (child and adult). In the dynamic video clip, actors are seated on chairs with a table in between, and four toy objects (house, hat, horse, bear) are presented in the background (center, top, left, right). The plot starts with the adult presenting a piece of chocolate to the child. The adult then nonverbally and verbally communicates to the child to wait and not to take the chocolate yet. The adult then places the chocolate in one of her closed hands, shows her closed hands and asks

the child to guess in which hand she's holding the chocolate. Once the child correctly identifies the hand with the chocolate, the adult shows the chocolate, but, unexpectedly, does not allow the child to take the chocolate. The child shows confusion and disappointment. See Figure 1 part A for a screenshot of the video clip.

In order to preserve ecological validity all sounds, including speech, were retained. In order to prevent interference from language abilities, language used in the clip was not the same as language of the participants (i.e., Italian versus Dutch), so none of the children were able to understand what was said. Dynamic AOIs were created for the two faces of the actors, which were taken together to obtain the AOI "Faces", and for the eyes of the actors, which were taken together to obtain the AOI "Eyes".

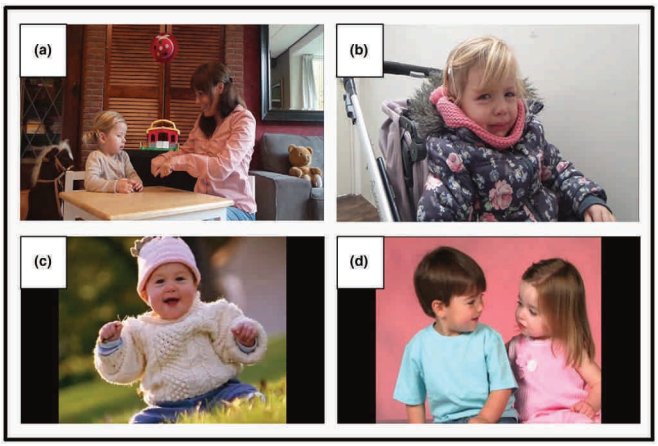

Figure 1. Screenshots of the dynamic video clips in the social interaction paradigm (a), facial emotion paradigm (b) and the single/multiple face paradigm multiple faces (c) and single faces (d)

Eye Tracking of Single/Multiple Faces

This paradigm consisted of two conditions: single faces and multiple faces. There were 6 blocks (3 single, 3 multiple) of 15 sec each, resulting in a total task time of 90 sec. The blocks were presented in alternate order (i.e., single, multiple, single, multiple, single, multiple). In each block a video dynamic clip was shown. In the Single face condition, there was only one face of a child on screen, in the Multiple faces condition there were always two or more faces (child-child or child-adult) on screen. The video clips were taken from the TV broadcasted series "Baby Einstein". See Figure 1 part C and D for screenshots of the video clips. There was no speech involved: The videos were accompanied by child-friendly instrumental music. Dynamic AOIs were manually created for 'Eyes' and 'Faces'.

Eye Tracking and Arousal Responses to Facial Emotions

This paradigm consisted of two conditions: happy facial emotion and sad facial emotion. There were 2 blocks (1 happy, 1 sad) of 30 sec each, resulting in a total task time of 60 sec. In each block a dynamic video clip was shown, taken from home-video movies displaying a child with genuine, real-life emotions. Sounds were retained in the clips, in order to have optimal ecological validity. There was no speech involved in the clips, except for the child saying

'mama'. The happy clip showed a child laughing and giggling while looking into the camera, the sad clip showed a child being upset and crying while looking into the camera. See Figure 1 part B for a screenshot of the video clip. Dynamic AOIs were manually created for 'Eyes' and 'Faces'.

Physiological Arousal: Heart Rate Measurements

Heart rate was measured during a resting state videoclip, and during the Emotional faces eye tracking paradigm. The resting state video clip was presented directly before starting the Emotional faces clip. It showed a relaxing cartoon (nature scenes accompanied by relaxing, classical music) with a duration of 3 minutes. Directly after the resting test, the Emotional faces test was started. The increase in heart rate from the resting state clip to the Emotional faces clip was used as a measure of emotional responsiveness to the emotional faces.

Heart rate was assessed based on the Electrocardiogram (ECG) signal, recorded continuously with a BIOPAC data acquisition system (MP150 Windows), using an Electrocardiogram amplifier (ECG100C), and AcqKnowledge software (Version 4.3.1. BIOPAC Systems Inc.). Acqknowledge software was synchronized with Tobii software by event markers representing the start of the video clip. Recording electrodes were placed at the top center of the chest (10 centimeters below the suprasternal notch), and at the bottom left and right of the ribs (10 centimeters above the bottom of the rib cage). The sampling rate was 200 Hz. In AcqKnowledge a 0.5 Hz highpass filter and a 50 Hz notch filter were applied to stabilize the ECG signal. Motion artifacts were visually identified and excluded from the data. The ECG signal was further processed by manually inspecting the detected R peaks and valid interbeat intervals (IBI) in MATLAB Release 2012b (The MathWorks, Inc., Natick, Massachusetts, United States). Based on the R peaks, heart rate (beats per minute, BPM) was obtained. Heart rate variability (HRV) was obtained by calculating the Root Mean Square of Successive Differences (RMSSD) of the interbeat intervals.

Study Procedures

For all participants signed informed consent was obtained from both parents. The study was approved by the ethical committees of Child and Education Studies at Leiden University and the Leiden University Medical Center. Testing was done in a quiet room at the University or at home. The laptop with the eye tracker was placed in a small semi-open tent to standardize the testing environment. The child was seated in front of the eye tracker. The examiner was seated behind the child (operating Tobii Studio using a remote keyboard), and the parent or caregiver was seated in the back of the room. The eye tracking session began with seating the child in the car seat in front of the eye tracker and placing the recording electrodes on the chest. After this, children watched a cartoon for 10 minutes to help the child get settled and to allow for arousal to reach a stable baseline level, without interference of any physical activity. After this, the calibration procedure for eye tracking started and the eye tracking clips were shown in fixed order (Single/Multiple faces, Social interactions, Emotional faces). The Emotional faces test was preceded by a neutral, resting state video clip to assess baseline levels of arousal. The structured observation task (ESCS) always took place after the eye-tracking session. The experimenter involved in the ESCS always had a fixed amount of interaction time with the child

before starting the test, in order to prevent familiarity differences to interfere with the test scores.

Results

Statistical Analyses

Statistical Package for the Social Sciences (SPSS) version 23 was used for statistical analyses. Effects of AOI in the eye tracking paradigm were tested using within subjects GLM, with the factor 'AOI' with two levels (faces, eyes). Paired samples T-tests were used for post-hoc analyses. In order to assess the association between eye tracking parameters and daily life social behavior, regression analyses were done with fixation duration to the AOIs as the dependent variables, and the following predictors: Vineland Socialization total score, SRS Social motivation, SRS Social cognition, SRS Social communication, SRS social awareness, ESCS Initiating social interactions, ESCS Initiating behavioral requests, and ESCS Initiating joint attention. The eye tracking data were used as dependent variables, because 1) the eye tracking variables show more and higher intercorrelations and were therefore less suitable to use as independent predictors, and 2) the social behavioral data consisted of a lower number of variables and thus smaller amount of predictors, leaving more statistical power in each regression model, which is relevant considering our limited sample size. According to power analysis (with 80 % power and the threshold for significance set at $p = .05$), the sample size of 32 children enabled detection of associations of at least $r = .47$.

Increases in BPM and HRV from rest to the emotional (happy and sad) conditions of the Emotional faces test were tested using paired samples T-tests. A delta score for BMP and HRV was calculated by subtracting the scores during rest from the scores during the happy or sad condition. These delta scores were used for correlational analyses. For all correlational analyses, Spearman's Rho was used. For GLM and regression analyses threshold for significance was set at $p = .05$. For correlational analyses the threshold was set at $p = .01$, to correct for multiple comparisons. All analyses were done based on the statistics handbook by Field (2013).

Intellectual Functioning

Mean FSIQ was 102.7 (SD 12.3), with a mean VIQ of 104.1 (SD 11.3) and a mean PIQ of 101.6 (SD 13.3).

Social Behavior

Mean scores for the social behavioral measures are presented in Table 2. All parameters were normally distributed in the sample, except for the Vineland Socialization total score and the ESCS initiating behavioral requests score, which showed some minor kurtosis. See supporting information for a correlation matrix of social behavioral measures with age, FSIQ, VIQ and PIQ.

Table 2. Means and standard deviations for scores on social behavioral measures

Measure	Mean
SRS total *T* score	46.3 (4.7)
SRS social awareness *T* score	50.6 (9.6)
SRS social cognition *T* score	47.1 (5.1)
SRS social communication *T* score	45.9 (5.2)
SRS social motivation *T* score	44.4 (5.9)
Vineland socialization total normscore	98.3 (6.6)
ESCS initiating joint attention	65.2 (16.8)
ESCS initiating behavioral requests	11.8 (6.9)
ESCS initiating social interaction	2.1 (1.4)

Abbreviations: ESCS: Early social communication scales; SRS: Social responsiveness scale ; Vineland: Vineland adaptive behavior scales

Eye Tracking

Eye Tracking of Social Interactions: Relation with Real Life Social Behavior

Data of one child were not included in the analyses because of extreme Z scores, resulting in a dataset of 31 children. The mean percentage time spent looking at the screen was 98.3 % (SD 0.9). The main outcome measures, i.e., proportion fixation duration for each of the AOIs, was not correlated with age, FSIQ, VIQ or PIQ (see supporting information) and did not show gender differences. Proportions of fixation duration for the AOIs 'eyes' and 'faces' are presented in Table 3.

Table 3. Proportions fixation duration (% fixation duration in proportion to the total visit duration toward the screen) for the various eye tracking tasks and AOI

Paradigm	AOI	Proportion fixation duration Mean (SD)
Social interaction	Faces	25.2 (9.8)
	Eyes	12.1 (9.8)
Single/Multiple faces	Single faces: Faces	60.0 (13.3)
	Single faces: Eyes	24.6 (10.9)
	Multiple faces: Faces	73.9 (15.2)
	Multiple faces: Eyes	15.1 (7.0)
Facial emotion	Happy: Faces	55.2 (11.6)
	Happy: Eyes	13.6 (7.4)
	Sad: Faces	56.9 (19.8)
	Sad: Eyes	20.6 (14.7)

In order to assess the association between eye tracking parameters and daily life social behavior, two regression analyses (see Figure 2) were done with the dependent variables proportion fixation duration in the AOI 'faces' and 'eyes', and the social behavioral measures as predictors. For the AOI 'faces', a significant model was found explaining 24.1 % of the variance ($F(3,27) = 2.8$, $p = .05$. This model contained three predictors: ESCS Initiating social interactions ($\beta = 0.35$, $t = 1.9$, $p = .06$) and Vineland Socialization total score ($\beta = 0.38$, $t = 2.1$, $p = .03$). In other words, increased attention to faces was associated with more social interactions and more adaptive social behavior in daily life. For the AOI 'eyes', a significant model was found explaining 21.7 % of the variance ($F(2,28) = 3.8$, $p = .03$. This model contained two predictors: ESCS Initiating social interactions ($\beta = 0.39$, $t = 2.2$, $p = .03$) and SRS Social cognition ($\beta = - 0.37$, $t = - 2.1$, $p = .03$. In other words, increased attention to eyes was associated with more social interactions, and fewer social cognition problems in daily life.

Eye Tracking of Single/Multiple Faces: Relations with Real Life Social Behavior

Data of one child were not included in the analyses because of extreme Z scores, resulting in a dataset of 31 children. The mean percentage time spent looking at the screen was 87.2 % (SD 7.8) for the Single face condition and 90.6 % (SD 5.6) for the Multiple face condition. The main outcome measures, i.e., proportion fixation duration for each of the AOIs, were not correlated with age, FSIQ, VIQ or PIQ (see supporting information), and did not show gender differences.

Proportions of fixation duration for the AOIs 'eyes' and 'faces' in the Single face and Multiple face conditions are presented in Table 3. In order to assess the association between eye tracking parameters and daily life social behavior, four regression analyses were done with the dependent variables fixation duration in the AOI 'faces' and 'eyes' in the Single face condition and Multiple face condition, and the social behavioral measure as predictors. In the Single face condition, no significant regression models were found for the AOIs 'eyes' or 'faces'. In the Multiple face condition, a significant regression model (covaried for age) was found for the AOI 'eyes', $F(1,29) = 5.1$, $p = .03$, which explained 15.0 % of the variance. This model contained one significant predictor: ESCS Initiating Behavioral Requests, ($\beta = - 0.38$, $t = - 2.2$, $p = .03$). In other words, increased attention to eyes in the multiple face condition was associated with more behavioral requests in social interactions. For the AOI 'faces' in the multiple face condition, no significant regression model was found. See Figure 2 for an overview of the associations between eye tracking variables and social behavior.

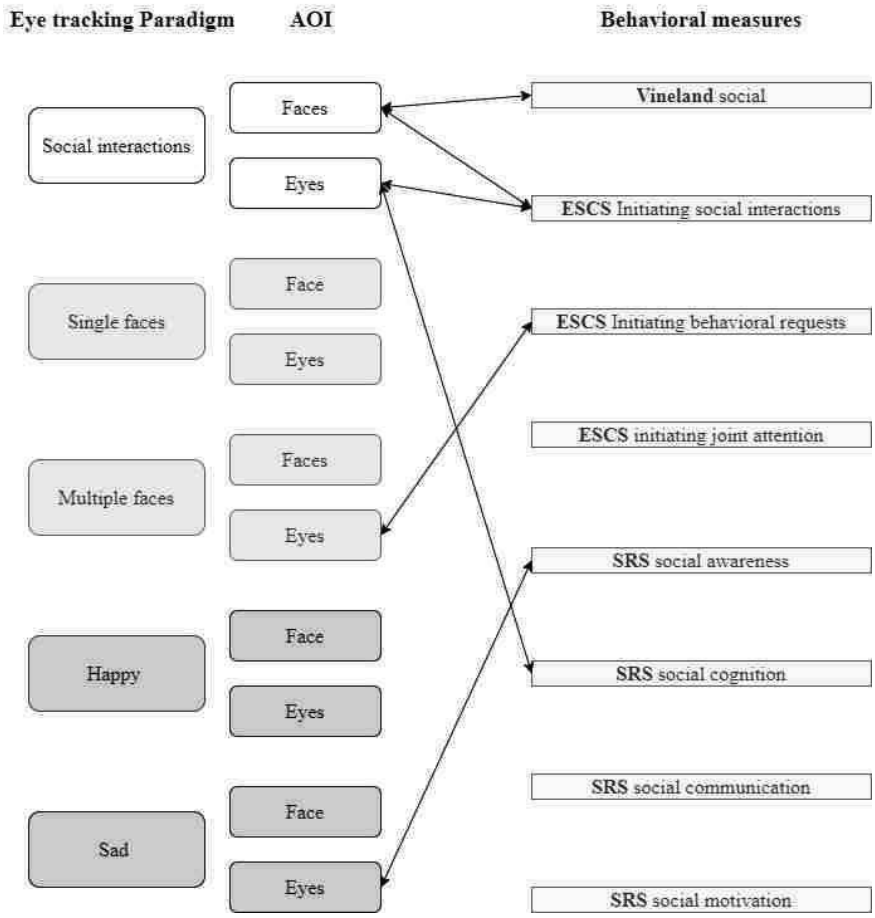

Figure 2. Overview of significant associations between fixation duration toward social cues in three eye tracking paradigms (blank, light grey, dark grey) with behavioral measures (observational, questionnaire, interview) of social adaptation. ESCS: Early social communication scales; SRS: Social responsiveness scale; Vineland: Vineland adaptive Behavior Scales

Eye Tracking of Facial Emotions: Relations with Real Life Social Behavior

For eye tracking analysis, data of 3 children were discarded because of incomplete data, resulting in a sample size of 29 children.

As for looking times during eye tracking, children attended to the screen for 95.7 % (SD 3.8) of the time in the happy condition and 95.1 % (SD 3.9) of the time in the sad condition. The main outcome measures, i.e., proportions of fixation durations for the AOIs 'eyes' and 'faces', were not significantly correlated with age, FSIQ, VIQ or PIQ (see supporting information), and did not show significant gender differences.

Proportions of fixation durations for the AOIs 'eyes' and 'faces' in the Happy and Sad conditions are presented in Table 3. In order to assess the association between eye tracking parameters and daily life social behavior, four regression analyses (see Figure 2) were done with the dependent variables proportions of fixation durations in the AOI 'faces' and 'eyes' in the happy condition and sad condition, and the social behavioral measures as predictors. For the AOI 'eyes' in the sad condition, a significant model was found, $F(1,28) = 4.2$, $p = .04$, with an explained variance of 13.2 %. This model contained one significant predictor, which was SRS Social Awareness, ($\beta = -0.36$, $t = -2.0$, $p = .04$). In other words, shorter looking times toward eyes in the sad condition were associated with more problems in social awareness. Other regression analyses did not result in significant models.

Arousal Responses to Facial Emotions

For physiology analyses, data of 9 children were discarded, due to children removing the electrodes ($n = 2$), extreme scores on the baseline rest measurement ($n = 4$), and motion artifacts ($n = 3$), resulting in a sample size of 20 children.

We first assessed if heart rate or HRV increased from the rest condition to the emotional (happy/sad) condition, in order to evaluate if emotional arousal was induced successfully by the task. As for HRV, values significantly increased from the rest condition ($M = 70.5$, $SE = 6.6$) to the happy condition ($M = 82.1$, $SE = 7.0$), $t(19) = -2.5$, $p = .01$. HRV values also significantly increased from the rest condition ($M = 70.5$, $SE = 6.6$) to the sad condition ($M = 83.0$, $SE = 7.1$), $t(19) = -2.0$, $p = .05$. See Figure 3. As for BPM, there was no significant increase from the rest condition ($M = 89.3$, $SE = 1.8$) to the happy condition ($M = 89.5$, $SE = 1.8$), $t(19) = -.35$, $p = .72$. In contrast, there was a significant increase from the rest condition ($M = 89.3$, $SE = 1.8$) to the sad condition ($M = 91.8$, $SE = 1.7$), $t(19) = -2.7$, $p = .01$. See Figure 3.

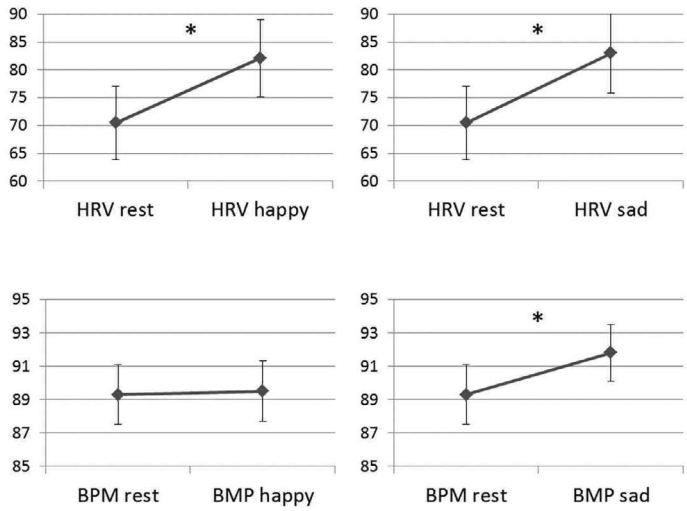

Figure 3. Average (and SE) scores for beats per minute (BPM) and heart rate variability (HRV) during rest and in response to happy and sad facial emotions

In order to interpret looking times toward facial emotions in relation to affective arousal in response to these stimuli, fixation duration for 'eyes' and 'faces' in the happy and sad conditions were entered in correlational analyses together with the amount of increase in BPM and HRV (the difference between rest condition and facial emotion condition). Results showed a significant correlation between the increase in HRV from rest to the sad condition and the proportion fixation duration toward 'eyes' in the sad condition ($r = -.53$, $p = .01$). In other words, stronger affective arousal responses in response to sad expressions were associated with shorter looking times toward eyes.

Discussion

This study was designed to evaluate to what degree eye tracking paradigms of social attention, in combination with psychophysiological measurements of affective arousal in response to social stimuli, are associated with real-life social behavior of young children.

Core to this study, we found multiple relationships between time spent looking at the eyes or face of a person and real-life social functioning as measured with structured behavioral observations by the experimenter (Early Social Communication Scales), as well as behavioral questionnaires (Social Responsiveness Scale) and interviews (Vineland Adaptive Behavior Scales) based on parent report. Social behaviors that were significantly associated with looking times included initiating social interaction, initiating behavioral requests, social awareness, social cognition, and overall social adaptive ability.

In exploring the type of social stimuli that were associated most strongly with real life social behaviors, we found that the relation between looking times in the eye tracking paradigms and the social behavioral measures varied according to specific type of stimulus. The paradigm involving social interactions showed the most relations, covering all of the social behavioral instruments including structured behavioral observations by the experimenter, as well as behavioral questionnaires and interviews based on parent report. In line with this, in the faces paradigm, attention to multiple faces did show relations with one of the social behavioral measures (structured behavior observations), but attention to single faces did not show any relations. In addition, we found that the area of interest 'eyes' showed significant relations with social behavioral measures in each of the three eye tracking paradigms. In contrast, the area of interest 'faces' was related to social behavior in only one of the three paradigms. Also, attention to happy faces was not associated with social behavioral measures, whereas sad faces did show relations with real life social functioning. Indeed, it has been suggested that particularly negative emotional expressions lead to more activation of the amygdala (Straube et al., 2008), a brain structure that plays a key role in social behavior (Adolphs, 2003). Based on our findings, future studies should preferably focus on stimuli that are dynamic and display multiple persons having social interactions (i.e., stimuli with higher levels of social complexity), negative emotional expressions, and the eye regions of the persons to be able to capture the social features that are most strongly associated with real life social behavior.

In order to better understand and interpret individual differences in looking times toward emotionally relevant social stimuli, affective arousal in response to the social stimuli was also studied. The paradigm we used, which involved dynamic video clips of facial expressions of

genuine (real-life) emotions, successfully triggered the autonomic nervous system as expressed in increased heart rate and heart rate variability. Sad facial expressions were more consistent in triggering increased arousal than happy facial emotions. Interestingly, stronger affective arousal responses in response to sad expressions were associated with shorter looking times toward eyes. In turn, shorter looking times toward eyes in the sad condition were associated with less social awareness in daily life. This pattern of findings suggests that some children may be overwhelmed by emotions of others and may not (yet) possess adequate emotion regulation strategies to successfully downregulate the increased arousal. Attentional deployment (e.g., avoidance) has proven to be less effective in the regulation of emotions than reappraisal strategies (Gross & Thompson, 2007). By diverting attention away from the eyes when emotions are in play, children may miss out on crucial information with regard to the feelings and intentions of others.

For typically developing children, an early social preference toward relevant social stimuli is typically largely automatic, and requires little effort (Rosa Salva et al., 2011; Simion et al., 2008). The degree to which children show spontaneous attention towards crucial social elements in the environment may have substantial impact on the foundation of social learning and the quantity as well as quality of social behaviors in daily life. This calls for sensitive and objective instruments to capture individual differences in social abilities in young children; eye tracking may prove to be a valuable addition to this. Although picking up on emotional expressions of others is important for successful social interactions, one's own emotions seem to play an equally important role in adaptive social behavior. Atypical arousal responses resulting from poor emotion regulation have been associated with lower quality of friendships, reduced interpersonal sensitivity, less prosocial tendencies and more social conflicts in young adults as well as reduced social adaptation and low peer friendship nominations in children and adolescents (Eisenberg et al., 2000; Halberstadt et al., 2001; Lopes et al., 2005; Mestre et al., 2006). In order to meet social goals in an adaptive way, it is necessary to have and maintain an optimum level of arousal, which helps in steering and tuning our behavior in social situations (Chambers et al., 2009; Mauss & Robinson, 2009). The relevance of studying biological parameters of arousal in children increasingly is becoming recognized because the degree to which social cues of others impact the autonomic nervous system might be fundamental to social development. Measuring heart rate in response to social stimuli may prove to be a helpful tool in assessing the fundaments of social development.

In terms of applicability, several factors were explored, including child characteristics (such as age, IQ and gender) and stimuli characteristics (such as type of stimulus). First of all, this study showed that the eye tracking paradigms were suitable for young children, ages 3 to 7 years. When considering the degree to which young children remained "on task" during the experiment, analysis of looking times showed that children were attending to the eye tracking screen as a whole for on average 98.3 %, 87.2 %, 90.6 %, 95.7 % and 95.1 % of the time, depending on the various eye tracking stimuli. These findings are relevant, considering that it is important to be able to keep children engaged with a task in order to obtain valid data. Keeping younger children engaged may be a challenge because they may have a shorter window of concentration as compared to older children. As eye tracking typically does not involve an experimenter who interacts with the child during testing (which for example is the case in

neurocognitive testing), it is crucial that the eye tracking stimuli by themselves are sufficiently engaging to allow for valid data collection. Furthermore, looking times toward regions of interest on the screen were overall not correlated with age, FSIQ, VIQ or PIQ, suggesting that the eye tracking stimuli can be used to assess and compare social attention in groups of young children who vary in age, and level of intellectual functioning, including performance IQ and verbal IQ. Eye tracking measures may especially be helpful in studies of clinical populations, in which intellectual functioning is often different from non-clinical control groups. However, a "minimum IQ" for such eye tracking paradigms remains to be identified. Also, none of the eye tracking paradigms showed differences in scores for boys versus girls, which indicates that they can be used in studies that have mixed samples of boys/girls.

The study also had several limitations. Considering the sample size, only a limited number AOIs in the eye tracking paradigms were analyzed; more AOIs would result in more levels in the multivariate analyses, and hence would require more statistical power. Also, the limited sample size did not allow us to identify subgroups with specific profiles or to calculate cut-off scores in eye tracking data. Replication in larger studies is needed, with a more diverse sample. The current study only included typically developing children, which is a limitation. Future studies are needed to assess the association between these eye tracking paradigms and social behavior in clinical groups. Stimuli were of high ecological validity but at the price of less experimental control to allow for more direct comparisons across paradigms. Also, data were collected only once, which did not allow for assessment of test-retest reliability.

Nonetheless, findings of this study suggest that looking patterns of children as measured with eye tracking are reflective of their real-life social behaviors, which may fuel implementation of sensitive and objective techniques in the study of early social development. Being able to orient spontaneously to social elements in the environment and to regulate emotions that are triggered adequately, is a prerequisite for socio-emotional development of children and is an important target in early treatment and intervention for children with severe disruptions in socio-emotional development (Bruinsma et al., 2004; Mazefsky & White, 2014). Eye tracking measurements are suitable for young children, children with varying levels of intellectual functioning, children with varying language abilities, and mixed groups of boys and girls. Thus, eye tracking paradigms, possibly in combination with psychophysiology, may provide opportunities to improve the evaluation of early intervention strategies targeting socio-emotional functioning, and to improve the extrapolation of effectiveness to real-life social abilities. Finally, our findings may stimulate new developments in individual assessment in young children with compromised socio-emotional development, for which eye tracking and psychophysiology is currently not (yet) available as part of clinical care.

References

Achenbach, T. M. (1991). *Manual for the Child Behaviour Checklist / 4-18 and 1991 profile*. Burlington VT University of Vermont Department of Psychiatry.

Adolphs, R. (2003). Cognitive neuroscience of human social behaviour. *Nature Reviews Neuroscience*, *4*, 165-178. https://doi.org/10.1038/nrn1056

Ames, C., & Fletcher-Watson, S. (2010). A review of methods in the study of attention in autism. *Developmental Review*, *30*(1), 52-73. https://doi.org/10.1016/j.dr.2009.12.003

Birmingham, E., Bischof, W. F., & Kingstone, A. (2008). Social attention and real-world scenes: The roles of action, competition and social content. *Quarterly Journal of Experimental Psychology*, *61*(7), 986-998. https://doi.org/10.1080/17470210701410375

Bruinsma, Y., Koegel, R. L., & Koegel, L. K. (2004). Joint attention and children with autism: A review of the literature. *Mental Retardation and Developmental Disabilities Research Reviews*, *10*, 169-175. https://doi.org/10.1002/mrdd.20036

Bruni, T. P. (2014). Test review: Social Responsiveness Scale–second edition (SRS-2). *Journal of Psychoeducational Assessment*, *32*(4), 365-369. https://doi.org/10.1177/0734282913517525

Campbell, J. M. (1998). Internal and external validity of seven Wechsler Intelligence Scale for Children - third edition short forms in a sample of psychiatric inpatients. *Psychological Assessment*, *10*, 431-434. https://doi.org/10.1037/1040-3590.10.4.431

Chambers, R., Gullone, E., & Allen, N. B. (2009). Mindful emotion regulation: An integrative review. *Clinical Psychology Review*, *29*, 560-572. https://doi.org/10.1016/j.cpr.2009.06.005

Chen, N. T. M., & Clarke, P. J. F. (2017). Gaze-based assessments of vigilance and avoidance in social anxiety: A review. *Current Psychiatry Reports*, *19*(9), Article 59. https://doi.org/10.1007/s11920-017-0808-4

Chevallier, C., Parish-Morris, J., McVey, A., Rump, K. M., Sasson, N. J., Herrington, J. D., & Schultz, R. T. (2015). Measuring social attention and motivation in autism spectrum disorder using eye-tracking: Stimulus type matters. *Autism Research*, *8*(5), 620-628. https://doi.org/10.1002/aur.1479

Chita-Tegmark, M. (2016). Social attention in ASD: A review and meta-analysis of eye-tracking studies. *Research in Developmental Disabilities*, *48*, 79-93. https://doi.org/10.1016/j.ridd.2015.10.011

Cicchetti, D. V., & Sparrow, S. A. (1981). Developing criteria for establishing interrater reliability of specific items: Applications to assessment of adaptive behavior. *American Journal of Mental Deficiency*, *86*, 127-137.

Constantino, J. N., Davis, S. A., Todd, R. D., Schindler, M. K., Gross, M. M., Brophy, S. L., . . . Reich, W. (2003). Validation of a brief quantitative measure of autistic traits: Comparison of the social responsiveness scale with the autism diagnostic interview - revised. *Journal of Autism and Developmental Disorders*, *33*, 427-433. http://doi.org/10.1023/a:1025014929212

Constantino, J. N., & Gruber, C. P. (2012). *Social Responsiveness Scale, second edition (SRS-2)* [Manual]. Western Psychological Services.

Dawson, G., Toth, K., Abbott, R., Osterling, J., Munson, J., Estes, A., & Liaw, J. (2004). Early social attention impairments in autism: Social orienting, joint attention, and attention to distress. *Developmental Psychology*, *40*(2), 271-283. https://doi.org/10.1037/0012-1649.40.2.271

De Bildt, A., Kraijer, D., Sytema, S., & Minderaa, R. (2005). The psychometric properties of the Vineland Adaptive Behavior Scales in children and adolescents with mental retardation. *Journal of Autism and Developmental Disorders*, *35*, 53-62. https://doi.org/10.1007/s10803-004-1033-7

Eisenberg, N., Fabes, R. A., Guthrie, I. K., & Reiser, M. (2000). Dispositional emotionality and regulation: Their role in predicting quality of social functioning. *Journal of Personality and Social Psychology*, *78*, 136-157. https://doi.org/10.1037/0022-3514.78.1.136

Falck-Ytter, T., Bölte, S., & Gredebäck, G. (2013). Eye tracking in early autism research. *Journal of Neurodevelopmental Disorders*, *5*, Article 28. https://doi.org/10.1186/1866-1955-5-28

Falck-Ytter, T., Fernell, E., Gillberg, C., & von Hofsten, C. (2010). Face scanning distinguishes social from communication impairments in autism. *Developmental Science*, *13*(6), 864-875. https://doi.org/10.1111/j.1467-7687.2009.00942.x

Field, A. (2013). *Discovering statistics using IBM SPSS Statistics* (4th ed. ed.). SAGE publications.

Frank, M. C., Amso, D., & Johnson, S. P. (2014). Visual search and attention to faces during early infancy. *Journal of Experimental Child Psychology*, *118*, 13-26. https://doi.org/10.1016/j.jecp.2013.08.012

Frank, M. C., Vul, E., & Saxe, R. (2012). Measuring the development of social attention using free-viewing. *Infancy*, *17*(4), 355-375 https://doi.org/10.1111/j.1532-7078.2011.00086.x

Gross, J. J., & Thompson, R. A. (2007). Emotion regulation: Conceptual foundations. In J. J. Gross (Ed.), *Handbook of emotion regulation* (pp. 3-24). Guilford Press.

Guillon, Q., Hadjikhani, N., Baduel, S., & Roge, B. (2014). Visual social attention in autism spectrum disorder: Insights from eye tracking studies. *Neuroscience and Biobehavioral Reviews*, *42*, 279-297. https://doi.org/10.1016/j.neubiorev.2014.03.013

Halberstadt, A. G., Denham, S. A., & Dunsmore, J. C. (2001). Affective and social competence. *Social Development*, *10*, 79-119. https://doi.org/10.1111/14679507.00150

Helminen, T. M., Kaasinen, S. M., & Hietanen, J. K. (2011). Eye contact and arousal: The effects of stimulus duration. *Biological Psychology*, *88*(1), 124-130. https://doi.org/10.1016/j.biopsycho.2011.07.002

Jones, W., Carr, K., & Klin, A. (2008). Absence of preferential looking to the eyes of approaching adults predicts level of social disability in 2-year-old toddlers with autism spectrum disorder. *Archives of General Psychiatry*, *65*(8), 946-954. https://doi.org/10.1001/archpsyc.65.8.946

Klin, A., Jones, W., Schultz, R. T., Volkmar, F., & Cohen, D. (2002). Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. *Archives of General Psychiatry*, *59*, 809-816. https://doi.org/10.1001/archpsyc.59.9.809

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biological Psychology*, *84*, 394-421. https://doi.org/10.1016/j.biopsycho.2010.03.010

Lopes, P. N., Salovey, P., Cata, S., & Beers, M. (2005). Emotion regulation abilities and the quality of social interaction. *Emotion*, *5*, 113-118. https://doi.org/10.1037/1528-3542.5.1.113

Louwerse, A., Van der Geest, J. N., Tulen, J. H. M., Van der Ende, J., Van Gool, A. R., Verhulst, F. C., & Graeves-Lord, K. (2013). Effects of eye gaze directions of facial images on looking behaviour and autonomic responses in adolescents with autism spectrum disorders. *Research in Autism Spectrum Disorders*, *7*, 1043-1053. https://doi.org/10.1016/j.rasd.2013.04.013

Lydon, S., Healy, O., Reed, P., Mulhern, T., Hughes, B. M., & Goodwin, M. S. (2016). A systematic review of physiological reactivity to stimuli in autism. *Developmental Neurorehabilitation*, *19*(6), 335-355. https://doi.org/10.3109/17518423.2014.971975

Mauss, I. B., & Robinson, M. D. (2009). Measures of emotion: A review. *Cognition and Emotion*, *23*, 209-237. https://doi.org/10.1080/02699930802204677

Mazefsky, C. A., & White, S. W. (2014). Emotion regulation: Concepts and practice in autism spectrum disorder. *Child and Adolescent Psychiatric Clinics of North America*, *23*, 15-24. https://doi.org/10.1016/j.chc.2013.07.002

McEvoy, R. E., Rogers, S. J., & Pennington, B. F. (1993). Executive function and social communication deficits in young autistic children. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *34*, 563-578. https://doi.org/10.1111/j.1469-7610.1993.tb01036.x

Mestre, J. M., Guil, R., Lopes, P. N., Salovey, P., & Gil-Olarte, P. (2006). Emotional intelligence and social and academic adaptation to school. *Psicothema*, *18 Suppl*, 112-117.

Mundy, P., & Neal, R. (2000). Neural plasticity, joint attention, and a transactional social-orienting model of autism. In L. Gidden (Ed.), *International Review of Research in Mental Retardation* (Vol. 20, pp. 139-168). Academic Press. https://doi.org/10.1016/s0074-7750(00)80009-9

Mundy, P., Sigman, M., & Kasari, C. (1990). A longitudinal study of joint attention and language development in autistic children. *Journal of Autism and Developmental Disorders*, *20*, 115-128. https://doi.org/10.1007/BF02206861

Nuske, H. J., Vivanti, G., & Dissanayake, C. (2014). Reactivity to fearful expressions of familiar and unfamiliar people in children with autism: An eye tracking pupillometry study. *Journal of Neurodevelopmental Disorders*, *6*, Article 14. https://doi.org/10.1186/1866-1955-6-14

Price, R. B., Siegle, G. J., Silk, J. S., Ladouceur, C., McFarland, A., Dahl, R. E., & Ryan, N. D. (2013). Sustained neural alterations in anxious youth performing an attentional bias task: A pupilometry study. *Depression and Anxiety*, *30*, 22-30. https://doi.org/10.1002/da.21966

Reynolds, G. D., & Roth, K. C. (2018). The development of attentional biases for faces in infancy: A developmental systems perspective. *Frontiers in Psychology*, *9*, Article 222. https://doi.org/10.3389/fpsyg.2018.00222

Risko, E. F., Laidlaw, K., Freeth, M., Foulsham, T., & Kingstone, A. (2012). Social attention with real versus reel stimuli: Toward an empirical approach to concerns about ecological validity. *Frontiers in Human Neuroscience*, *6*, Article 143. https://doi.org/10.3389/fnhum.2012.00143

Rosa Salva, O., Farroni, T., Regolin, L., Vallortigara, G., & Johnson, M. H. (2011). The evolution of social orienting: Evidence from chicks (Gallus gallus) and human newborns. *PLoS One*, *6*, Article e18802. https://doi.org/10.1371/journal.pone.0018802

Salley, B., & Colombo, J. (2016). Conceptualizing social attention in developmental research. *Social Development*, *25*(4), 687-703. https://doi.org/10.1111/sode.12174

Seibert, J. M., Hogan, A. E., & Mundy, P. C. (1982). Assessing interactional competencies: The early social-communication scales. *Infant Mental Health Journal*, *3*. https://doi.org/10.1002/1097-0355(198224)3:4<244::AID-IMHJ2280030406>3.0.CO;2-R

Simion, F., Regolin, L., & Bulf, H. (2008). A predisposition for biological motion in the newborn baby. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 809-813. https://doi.org/10.1073/pnas.0707021105

Sparrow, S. S., Cicchetti, D. V., & Balla, D. A. (2005). *Vineland-II Adaptive Behavior Scales: Survey forms manual*. AGS Publishing.

Speer, L. L., Cook, A. E., McMahon, W. M., & Clark, E. (2007). Face processing in children with autism: Effects of stimulus contents and type. *Autism*, *11*(3), 265-277. https://doi.org/10.1177/1362361307076925

Stagg, S. D., Davis, R., & Heaton, P. (2013). Associations between language development and skin conductance responses to faces and eye gaze in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *43*, 2303-2311. https://doi.org/10.1007/s10803-013-1780-4

Straube, T., Pohlack, S., Mentzel, H. J., & Miltner, W. H. (2008). Differential amygdala activation to negative and positive emotional pictures during an indirect task. *Behavioural Brain Research*, *191*, 258-288. https://doi.org/10.1016/j.bbr.2008.03.040

Wechsler, D. (2002). *WPPSI-III: Technical and interpretative manual* The Psychological Corporation

Zantinge, G., Van Rijn, S., Stockmann, L., & Swaab, H. (2017). Psychophysiological responses to emotions of others in young children with autism spectrum disorders: Correlates of social functioning. *Autism Research*, *10*, 1499-1509. https://doi.org/10.1002/aur.1794

Chapter 8

Summary and Discussion

A *neurogenetic approach* can further our understanding of how neurocognitive mechanisms serve as building blocks for neurodevelopmental outcomes. By studying populations with neurobehavioral phenotypes associated with genetic disorders, ultimately individual 'at risk' patterns of development can be identified. Sex chromosome trisomy (SCT), a genetic disorder caused by the presence of an extra X or Y chromosome, is an interesting candidate for studying neurobehavioral phenotypes for several reasons. First, with a prevalence of 1:650 to 1:1000 live births, SCT is not rare, but one of the most common genetic duplications in humans. Second, in contrast to many other genetic disorders, global intellectual functioning in individuals with SCT is often within the normal range, thus interpretation of results is not limited. Third, SCT can be diagnosed prenatally, providing the opportunity to study development early and prospectively. Lastly, the X and Y chromosomes have not only been identified to play a role in neurodevelopment, but the prevalence of neurodevelopmental disorders such as ASD and ADHD is increased in individuals with SCT as well, indicating that SCT can be an important model to further our understanding of mechanisms that contribute to psychopathology.

So far, the majority of studies on SCT have focused on physical and medical consequences of the extra chromosome. Studies looking into the neurocognitive profile are more scarce. Thus, more studies are needed that look into the neurocognitive strengths and weaknesses of this population and how these strengths and weaknesses are related to behavioral outcomes. Within these studies, developmental impact should be taken into account for two reasons. First, early neurocognitive functioning can be a precursor for later development. As SCT can be diagnosed prenatally, early developmental impact can be investigated, providing the opportunity to learn more about the early pathways to psychopathology. Second, as (precursors of) neurocognitive functions start to develop early in life due to rapid brain development, the brain is more susceptible to interventions early in life; there are certain moments or *windows of opportunity* to alter the developmental pathway.

The studies in this dissertation aimed to gain more knowledge of the early development of young children with SCT within the behavioral, language, and communication domains. In addition, studies in this dissertation aimed to prospectively investigate the relationship between communication and behavioral outcomes, or in other words, to identify building blocks of behavioral outcomes. All studies were conducted in young children within the 1-7 year age-range.

When looking at the early presentation of behavioral outcomes – considering the risk for psychopathology in later development – the results in this thesis reveal a higher incidence of behavioral problems in young children with SCT. Further examining age-specific behavioral profiles illustrate that some behavioral outcomes, such as social-emotional difficulties can already be present in very young children (1-year of age), with other behavioral problems gradually emerging in older age groups. Also, the developmental pathway of some of the behavioral outcomes – although examined cross-sectionally rather than longitudinally – appears to be different in children with SCT compared to their peers **(Chapter 2)**. Four neurocognitive domains were identified as possible underpinnings of behavioral outcomes, namely global intellectual functioning, social cognition, executive functioning, and language and communication **(Chapter 3)**. Moreover, as language has been described as one of the core

neurocognitive vulnerabilities in the SCT population, knowing that language and communication are foundation skills that are needed for many other neurocognitive functions, and as difficulties with language and communication are associated with adverse behavioral outcomes and neurodevelopmental problems, the focus of this dissertation was on the language and communication domain. The results of **Chapter 4, 5, and 6** illustrate that language and communication difficulties can already be seen in very young children with SCT and that multiple communicative functions are vulnerable. Language difficulties can already be present before children start to use spoken words to communicate (**Chapter 4**), and communicative difficulties extend past structural language, as the use of language in a social setting (i.e., pragmatic language; **Chapter 5**) and social orientation and arousal modulation during communicative interaction also appeared to be affected **(Chapter 6)**. In addition, language and communication outcomes were relevant in predicting a variety of behavioral outcomes over time **(Chapter 4**) and attention to socially relevant cues, such as the eyes or mouth of an on-screen partner, was related to both concurrent and future language ability in very young children with SCT **(Chapter 6)**. Lastly, social orientation and arousal modulation were related to real-life social behavior in children from a population sample (**Chapter 7**), illustrating that these early functions are meaningful for daily life functioning. Taken together, early communicative abilities are associated with behavioral outcomes, therefore early monitoring of these abilities is important as early childhood may be an important time to positively influence further development.

Within the next paragraphs, the main findings of these studies are summarized and discussed. Finally, a general discussion, implications of the findings, and suggestions for future research will conclude this dissertation.

Summary

The Behavioral Profile of Young Children with SCT

The study presented in **Chapter 2** aimed to describe the early behavioral profile of young children with SCT and to identify if the presentation of the behavioral profile was age dependent. When including children between the ages of 1-5 years, higher incidences of social-emotional functioning problems, affective behavior problems, and pervasive developmental problems were reported in the SCT group compared to the control group. Risk assessment showed high variability within the SCT group; some children showed no behavioral problems, whereas others showed behavioral problems at a clinical level. Compared to the control group, children with SCT more often had a clinical or 'at-risk' score for social-emotional problems (40%), affective problems (11%), anxiety problems (16%), and pervasive developmental problems (38%). Further exploring behavioral outcomes in three age groups revealed age-dependent behavioral profiles. In 1-year-old children with SCT, difficulties with social-emotional functioning could already be present, and elevated scores were persistent across the 1–5-year-old age range. Affective and pervasive developmental behaviors were seen in 3-year-olds, and more prominent in 4-5-year-olds. Anxiety, attention deficit, and oppositional defiant behaviors were seen in 4-5-year-olds. Moreover, the development of affective, pervasive developmental, and oppositional defiant behaviors – although examined cross-sectionally rather than longitudinally – was different for children with SCT compared to the control group. Social-emotional problems however, appeared to be more stable and persistent within the entire

age range. Taken together, these results show that children with SCT are at risk for suboptimal behavioral development from a young age; a risk that appears to increase and expand across behavioral domains with increasing age.

Identifying Neurocognitive Domains as Building Blocks for Behavior

Through a narrative review of the literature, possible neurocognitive underpinnings of behavioral vulnerability were identified in **Chapter 3.** Evidence for cognitive impairment in the domains of global intellectual functioning, language development, executive functioning, and social cognition was evaluated. The aim of this narrative review was two-fold: First, to review existing literature to identify gaps in research that should be explored further and second to identify risk-factors that could serve as a potential target for support and intervention. Earlier reviews have focused on the development of individuals with SCT over the lifespan, primarily during adolescence and adulthood. Therefore, the focus of this review was on early development.

The results of this narrative review illustrate the need for studies in young children, in particular longitudinal studies to follow the developmental trajectory over time. Within the global intellectual functioning domain outcomes vary greatly, ranging from impaired to above average. Taking the results of eight studies together, global intellectual functioning of children with SCT is estimated to be in the average to low-average range. Within the language domain, language difficulties have been identified in young children and appear to be persistent. Taking results of five studies together, effect sizes indicated high clinical significance (i.e., Cohen's d > 1.00), stressing the importance of early detection and support of this domain. Within the executive functioning domain, outcomes were variable depending on the assessed function. Taking the results of five studies together, effect sizes ranged from low to high clinical significance. Finally, on the social cognition domain, taking results from six studies together, results indicated medium to high clinical significance.

To conclude, the results of this narrative review illustrate a significant gap in research focusing on the early developmental impact of SCT. Nonetheless, the existing literature hints that the presence of an extra X or Y chromosome impacts neurocognitive functioning. Knowledge of early development on these domains should be expanded to improve clinical care and to help identify targets for early support and intervention programs.

Early Language and Communication Abilities of Children with SCT

As language has been identified as a vulnerable neurocognitive domain in individuals with SCT, language and communication skills develop early in life, and language and communication are important for other neurocognitive functions, three studies in this dissertation focused on the early language and communication abilities of children with SCT. The study presented in **Chapter 4** aimed to identify language abilities in children with SCT at different developmental stages within the 1–6-year age range and to describe the variability of language development with clinical classifications. Regarding the language abilities at different developmental stages, our results showed that, compared to an age-matched control group, one-year-old children with SCT produce and understand fewer words, and have poorer receptive and expressive semantic skills. The three- to four-year-old children with SCT in our sample have similar receptive semantic and receptive syntactic language skills compared to children in the control group, but poorer expressive semantic skills. Lastly, in the five- to six-year-old children with SCT, we found poorer receptive semantic, expressive semantic, and receptive syntactic language skills.

Regarding the clinical classifications, our results showed much variability in language abilities, with rates of clinically relevant difficulties ranging from 12 to 50%. Odds ratio indicated that the risk of language difficulties was 2-7 times higher in the SCT group, depending on the language function. The study presented in **Chapter 5** further investigated how children use language in social settings (i.e., pragmatic language). Our results showed that children with SCT between the ages of 3–7 years experience more difficulties with all three investigated aspects of pragmatic language: Nonverbal communication, conversational routines, and requesting, giving, and responding to information. These difficulties were not only present in children with structural language problems but appeared to be a more common characteristic within the SCT group. Also, we found that the risk of having inadequate pragmatic language abilities was 23 times higher in the SCT group compared to the population sample. Lastly, the study presented in **Chapter 6** aimed to increase knowledge of how young children with SCT respond to short periods of communicative interactions with a dynamic eye tracking paradigm and physiological arousal measures. Our results indicate that children with SCT aged 1-7 years orient less to social aspects during communicative interactions, in particular to the eyes of the on-screen communicative partner. In addition, the group of children with SCT did not modulate their arousal levels in reaction to different situational demands (i.e., a change in gaze direction). Results of this study hint that children with SCT may experience difficulties with social communication that extend past the risk for early language delays.

Taking these results together, results of these studies add to the growing body of literature on language and communication difficulties in the SCT population. More importantly, these studies expand this knowledge by specifically focusing on young children, by examining specific language outcomes, and by exploring skills in the broader communication domain. As our results show that language and communication difficulties are present across early developmental stages and various skills within this domain are affected, it is likely that these difficulties are anchored in early brain development.

Associations between Language, Communication, and Behavior – Concurrent and Future Outcomes

In three studies, we further looked into associations between language, communication, and behavior and aimed to unravel which language and communication functions possibly serve as building blocks for behavioral outcomes and to gain knowledge of associations between language, communication, and behavior. In **Chapter 6** we investigated the relation between social orientation towards the face, eyes, and mouth of an on-screen communicative partner and both concurrent and future receptive and expressive language abilities. Our results showed that in one-year-old children with SCT both concurrent and future language skills on the receptive and expressive domain were positively correlated to time spent looking at the mouth. These results illustrate that social orientation and language are intertwined at a very young age. The results presented in **Chapter 7** aimed to evaluate to what degree social orientation and arousal modulation in response to social stimuli are associated with real-life social behaviors of young children from a population sample. Results of this study illustrate that social orientation, in particular attention to eyes, is related to real-life social behaviors such as initiation of social interaction, initiation of behavioral request, social awareness, social cognition, and overall social adaptive ability. The ‘social load’ of the paradigms played a role; the paradigm with the highest social load (i.e., a paradigm with social interaction) was related to more real-life behavioral outcomes than paradigms with lower social loads (i.e., paradigms with one person

or several persons without interaction). In addition, a strong physiological arousal response was associated with less social orientation to the eyes and subsequently with less social awareness in real life. Lastly, the results presented in **Chapter 5** illustrate the relevance of structural and pragmatic language on later behavioral outcomes. Poorer pragmatic and structural language abilities were predictive of more attention deficit problems, more pervasive developmental problems, and more social-emotional problems one year later. Poorer pragmatic language was also predictive of more affective problems and more oppositional defiant problems. Thus, pragmatic language in particular was predictive of a broad range of outcomes; social communicative abilities can serve as an early sign of later behavioral problems and may also help explain the variance in neurobehavioral outcomes.

Taking the results of these studies together, these results hint at a broader communication deficit in children with SCT that encompasses several tools that are needed to navigate the social world and that at least some of these communicative tools act as building blocks for later neurobehavioral outcomes.

General Discussion

The central aim of this dissertation was to gain knowledge of early language abilities of young children within the broader communication domain and to prospectively investigate the relationship between language, communication, and behavioral outcomes. From the studies included in this dissertation, four main conclusions can be drawn.

Conclusion 1: Communication Difficulties that Extend Language Vulnerabilities

Language is a vulnerable cognitive domain in children with SCT, but children with SCT may experience difficulties with communication that extend language abilities. Several language and communicative functions may be involved, including functions regarding the form and content of language, the use of language as a social tool, and the ability to rely on a 'social compass' which is needed to navigate social communicative interactions. Both comprehension (i.e., receptive abilities) and production (i.e., expressive abilities) can be affected. Language plays an important role in cognitive and social development (Simms, 2007), and is required to communicate one's needs, thoughts, and emotions. Language and communication are also needed for learning, reflecting on experiences, and to understand the world around us. As language and communication are intertwined with many other functions, compromised language and communication abilities could have severe consequences for the development of other neurocognitive functions and behavioral outcomes, consequently also affecting one's ability to participate in society or one's experienced quality of life.

Conclusion 2: Language and Communication as Building Blocks for Neurobehavioral Risk

Already at a young age, children with SCT have an increased risk for a range of neurobehavioral problems. This finding adds to the growing body of literature that individuals with SCT have an elevated risk for serious behavioral problems. Behavioral problems have been negatively associated with many other outcomes, such as daily life functioning, social competence, school performance, and peer acceptance (de Lijster et al., 2019). Also, behavioral problems during early childhood could be predictive of later psychopathology (Goodwin et al., 2004; Ormel et al., 2015; Roza et al., 2003). This illustrates the importance to further unravel which mechanisms underly this neurobehavioral risk and signal these 'at-risk' developmental trajectories. By targeting these mechanisms early on, this could hopefully reduce the risk of

more serious psychopathology in later life. Studies included in this dissertation illustrate that language and communication are neurocognitive building blocks that – at least in part – may drive, or present as early markers of, this increased risk. Vulnerable language and communicative abilities could lead to, or precede, various adverse behavioral outcomes; this stresses the importance to look into preventive support and to study if improving language and communication could also positively impact behavioral outcomes.

Conclusion 3: A Developmental Perspective is Key

A developmental perspective is key to understand the impact of SCT on both neurocognitive and behavioral outcomes. The studies presented in **Chapters 2, 4, 5, and 6** all included young children with SCT to increase our knowledge of the early development of this group. The majority of previous studies focused on school-age children, adolescents, and/or adults; thus, the results of the studies included in this dissertation fill an important gap in knowledge. In addition, increasing our understanding of the development and the developmental risk of language and communication in the SCT population could not only help to better serve the SCT population, but may also serve as a model to understand 'at-risk' pathways in general child development. In contrast to studies including children with a behavioral diagnosis, where early development can only be studied retrospectively, studying a group of children with a genetic diagnosis provides a unique opportunity to prospectively study the early markers and pathways of an 'at risk' development, such as seen in the earliest forms of communicative development.

To improve our understanding of early development, age was included as a factor to interpret results either by studying outcomes in specific age ranges or by investigating the developmental pathway of outcomes based on cross-sectional data. Results from **Chapters 2, 4, 5, and 6** illustrate that children with SCT have an increased risk for social-emotional problems, even as young as one-year-olds. In addition, in the communication and language domain, children with SCT may understand and use less words, may experience difficulties with other semantic abilities, and may rely less on tools such as social orientation and arousal modulation during interactions. In 3–4-year-old children, affective and pervasive developmental problems become more apparent, in addition to the social-emotional problems. In the language and communication domain, children may experience difficulties with expressive semantics, with using language in a social setting, and in using social tools during interaction. Finally, in children aged 5-7 years, children with SCT have an increased risk for social-emotional, affective, anxiety, pervasive developmental, attention deficit, and oppositional defiant problems. In the language and communication domain, receptive and expressive semantic skills, syntactic abilities, and pragmatic language may be affected, as well as social orientation and arousal modulation.

Our findings illustrate that language and communication abilities can already be affected from a young age. Results from neuroimaging studies suggest that brain anatomy and function may be impacted by the presence of an extra X or Y chromosome (Brandenburg-Goddard et al., 2014; Bryant et al., 2012; Giedd et al., 2007; Lenroot et al., 2014; Lentini et al., 2013; Nadig et al., 2018; Patwardhan et al., 2002; Raznahan et al., 2016; van Rijn et al., 2008; van Rijn et al., 2012; Warling et al., 2020). The early impact of SCT on language and communication abilities combined with the results of neuroimaging studies fit with the idea that these difficulties are anchored in early brain development. In the first years of life the brain develops rapidly; not only does the volume of the brain more than double within this time period, structural and functional networks increase tremendously as well. Although the brain continues

to mature, this occurs at a much slower pace compared to the development during early childhood (Gilmore et al., 2018). Due to this rapid brain development in the early years of life, the brain is more susceptible and early timing of interventions may help modify suboptimal development to a greater extent than later in development. As the risk for suboptimal development in children with SCT is present early in life *and* as for some functions this risk appears to increase and expand when children get older, this stresses the importance of early monitoring and interventions that could possibly influence the developmental trajectory of children with SCT in a positive manner.

Conclusion 4: Robust Vulnerabilities

Our studies did not find evidence that specific SCT characteristics such as karyotype, time of diagnosis, or ascertainment bias (i.e., the reason for participation in the study) play a significant role in explaining outcomes. In **Chapters 2, 4, 5, and 6,** we explored if these characteristics impacted results. Regarding the SCT karyotypes there were some differences in behavioral profiles when comparing children in the SCT group with their same-sex peers (i.e., XXX vs XX, XXY vs XY, XYY vs XY), but affective and social-emotional problems appeared to be persistent across variants. Our results did not indicate differences in the language and communication abilities of girls with an extra X, boys with an extra X, or boys with an extra Y. Regarding time of diagnosis, the behavioral profile of children with a postnatal diagnosis was more severely affected, which was expected as genetic testing may have been conducted due to behavioral problems. Social emotional problems, however, were also present in children with a prenatal diagnosis. Our results did not indicate differences in the language and communication abilities of children with a prenatal diagnosis versus children with a postnatal diagnosis. Lastly, language and communication abilities and behavioral profiles were not different between children who enrolled into the study as part of the prospective follow-up, information seeking, or clinically referred group. Taken together, the presence of an extra X or Y chromosome by itself has a greater impact on language, communication, social-emotional, and behavioral outcomes than specific SCT characteristics, such as karyotype, time of diagnosis, or ascertainment bias; the vulnerabilities identified in our studies appear to be robust within the SCT group.

Clinical Implications

Results of this dissertation illustrate that as a group, children with SCT have an increased vulnerability for both adverse behavioral outcomes and language and communication difficulties. However, the results also indicate that both behavioral outcomes and language and communication abilities are highly variable in children with SCT; some children may be severely affected where others are less affected or may not noticeably differ from peers. It should be noted that the results presented in this dissertation represent the average group of children with SCT, whereas all children are unique, and every child develops at his or her own pace. Based on the results of this dissertation, three clinical implications can be drawn.

Implication 1: Importance of Early Monitoring and Identification

We stress the importance of monitoring the broader communication domain in addition to language in routine clinical care and stress the early identification of adverse behavioral outcomes. When a child does not meet the age-appropriate milestones, standard neuropsychological screening is advised rather than a ‘wait and see’ approach. The standard for neuropsychological screening should be comprehensive, for example also including the earliest

stages of nonverbal communication and social aspects of communication. The developmental trajectory should be monitored closely as language and communication develop rapidly at a young age and the risk for adverse behavioral outcomes seems to increase when children get older.

Implication 2: Importance of Early Preventive Support or Intervention

Developing communication and language skills is an important task for young children and difficulties in the acquisition of these skills can have an impact on many other outcomes. Results from this dissertation identified social communication as an important marker for a range of neurobehavioral outcomes and strong associations between language and social orientation at a very young age, both concurrently and one year later were found. These findings stress the importance to provide preventive support or to intervene as early as possible. Early development is an important window of opportunity in which effective intervention can be crucial to ensure positive social and academic outcomes in later life (Kaiser & Roberts, 2011). It is important to investigate the effectiveness of existing evidence-based support and intervention programs for children with SCT, and if these programs do not suffice, to develop programs tailored to the specific needs of children with SCT.

Implication 3: Importance of Informing Professionals and Parents

Professionals should be aware of the possible impact of SCT, the role of 'expert' should not fall onto the parents' shoulders (Richardson et al., 2021). It is important that professionals such as genetic counselors, pediatricians, developmental psychologists, speech-and language pathologists, and physical therapists stay up to date on the knowledge of genetical conditions such as SCT.

Professionals should be aware of the wide variability in outcomes and should have knowledge of which domains to monitor even more closely at certain developmental stages. Professionals should also inform parents of this variability in outcomes when their child receives the diagnosis. Parents in turn, can provide valuable information to professionals: Parents' concerns are an important marker for early detection of neurocognitive or behavioral problems (Glascoe & Dworking, 1995).

Professionals should inform parents about early opportunities to stimulate development that parents can easily implement themselves. For example, to stimulate the language development of a child, parents are advised to read books with their child. This is a general recommendation that is important for all children but could possibly be crucial for children with an increased vulnerability for language difficulties, such as children with SCT. Furthermore, it is important that professionals provide parents with knowledge and tools how to support their child's *individual* needs so parents can create a safe and sensitive environment for their child to develop.

Strengths and Limitations

Language and communication are vulnerable neurocognitive domains in children with SCT that may be important to signal 'at-risk' developmental trajectories and can – at least in part – explain neurobehavioral outcomes. Unfortunately, the number of studies that have investigated neurocognitive and neurobehavioral functioning in SCT, especially in young children, is limited. The studies included in this manuscript were designed to gain knowledge in these domains. A major strength of the studies included in this manuscript was the sample; we were

able to include a large international sample of young children with SCT and because of this large sample size, we were able to look into age-specific outcomes and the influence of SCT characteristics such as karyotype, time of diagnosis, and ascertainment bias. In addition, the availability of behavioral outcomes one year later allowed us to predict behavioral outcomes over time. There were, however, also limitations to this study. In our study, we included children into two protocols: A protocol for one-year-old children and a protocol for children aged 3-7 years. In order to prevent mixing of these protocols within one individual child, two-year-old children were not represented in the study. Also, some measures were age-specific, therefore children of a certain age had to be excluded for some analyses. Lastly, recruitment bias will always lead to variance in the SCT phenotype, where some difficulties may be overestimated, and others may be underestimated. By including children with SCT regardless of time of diagnosis and reason for enrollment, we have attempted to reduce this bias. However, as not all families with a child with SCT opt to enroll in scientific studies and genetic testing may not always be performed, it cannot be excluded that the described outcomes are not fully representative for the total population of children with SCT.

Recommendations for Future Research

Based on the results of the studies included in this manuscript, there are a number of directions we recommend for future research.

First, as language and communication develop rapidly in the first years of life, we recommend expanding the age range and to study outcomes over a longer period of time. As children with SCT can be diagnosed prenatally, monitoring would preferably take place soon after birth. By studying development of neurocognitive functions across a longer time span and by projecting outcomes over a longer time period, the understanding of different pathways and factors that drive or moderate these pathways could increase.

Second, more knowledge is needed to gain insight into the overall neurocognitive profile of children with SCT and how neurocognitive functions relate to behavioral outcomes. This includes other functions in the language and communication domain but based on vulnerabilities that have been identified in older cohorts with SCT, more knowledge of early social cognitive functioning and executive functioning in young children with SCT is also warranted. In addition, studies that investigate how these neurocognitive functions relate to behavioral outcomes or to each other are scarce. It is possible that vulnerabilities on neurocognitive functions and behavioral difficulties may be due to deficits in metacognitive control functions. An example of such a function is self-directed speech. Self-directed speech emerges in the toddler years; toddlers will talk to themselves out-loud (i.e., overt). Gradually, this progresses to more covert speech, for example children will whisper to themselves, especially when performing a difficult task. Finally, self-directed speech will take on the form of inner speech (Mulvihill et al., 2020). Studies have shown than impaired self-directed speech can result in significant cognitive and behavioral impairments in both typically and atypically developing children and adults (Alderson-Day & Fernyhough, 2015; Whitehouse et al., 2006). Studies including children with neurodevelopmental disorders, for example developmental language disorder, ASD, and ADHD have suggested atypical development of self-directed speech (for an overview see Mulvihill et al., 2020). Due to the increased prevalence of neurodevelopmental disorders and the increased risk of neurocognitive difficulties, it could be of interest to study the concept of self-directed speech in individuals with SCT as well.

Third, development of children is dynamic; there is a complex relation between environmental and interpersonal factors, neurocognitive functions, and behavioral outcomes. More knowledge is needed about the impact of environmental and interpersonal factors. For example, language richness of the environment may serve as a risk- or protective factor in the development of language and communication. Interpersonal factors, for example services a child received and at what age may also impact later outcomes. This also includes the effect of testosterone replacement therapy. Effects of these environmental and interpersonal factors on neurocognitive and behavioral outcomes should be explored further.

Lastly, the effectiveness of existing intervention programs should be evaluated for children with SCT. Results from studies included in this dissertation show that social communicative abilities in particular are an important marker to identify children at risk for unfavorable outcomes, which could possibly also be related to risk for more severe psychopathology later in life. Interventions that are used in other populations, for example in children with autism spectrum disorder or with specific language impairment, and that target this neurocognitive building block could also be of interest for children with SCT. If existing intervention programs are not appropriate or do not exist, specific interventions tailored to the needs of children with SCT should be developed.

Conclusions

The studies included in this dissertation demonstrate that children with SCT have an increased vulnerability for adverse neurobehavioral outcomes and an increased risk for neurocognitive difficulties in the language and communication domain, starting from a young age. This risk for language and communication difficulties and vulnerability for adverse neurobehavioral outcomes may increase when children get older. Moreover, these neurocognitive functions appear to serve as early markers of at-risk pathways with unfavorable neurobehavioral outcomes. These results come with important clinical implications for the SCT population and will ideally fuel the implementation of early monitoring, and implementation and development of preventive support and intervention. Lastly, studying underlying mechanisms of adverse outcomes via a *neurogenetic* approach furthers our understanding of brain-behavior relationships in general.

Nederlandse Samenvatting en Discussie
Summary and Discussion in Dutch

Om meer kennis te vergaren hoe neurocognitieve mechanismen dienen als bouwstenen voor (neuro)ontwikkelingsuitkomsten, kan een *neurogenetische benadering* ingezet worden. Hierbij worden gedragsfenotypes in beeld gebracht die worden geassocieerd met genetische aandoeningen, om zo uiteindelijk 'risicopatronen' in de ontwikkeling te kunnen identificeren op individueel niveau. Geslachtschromosomale trisomie (*sex chromosome trisomy*; afkorting SCT), een genetische aandoening veroorzaakt door de aanwezigheid van een extra X of Y chromosoom, is in dat opzicht een interessante genetische aandoening waarbij gedragsfenotypes onderzocht kunnen worden om genetische impact beter te begrijpen en om mechanismen van risico te onderzoeken. SCT is met een prevalentie van 1:650 tot 1:1000 geboren kinderen niet zeldzaam. Sterker nog, SCT is een van de meest voorkomende genetische duplicaties bij mensen. Daarbij valt het globaal niveau van intellectueel functioneren bij individuen met SCT, in tegenstelling tot bij veel andere genetische aandoeningen, vaak binnen het normale bereik, waardoor resultaten gegeneraliseerd kunnen worden naar een bredere populatie. Ook kan SCT prenataal vastgesteld worden. Dit creëert de mogelijkheid om de ontwikkeling vroeg en prospectief te bestuderen. Tot slot spelen de X en Y chromosomen niet alleen een rol in de (neurobiologische)ontwikkeling; ook gedragsproblemen komen vaker voor bij SCT, wat bijvoorbeeld wordt geïllustreerd door het verhoogde percentage van symptomen van ontwikkelingsstoornissen zoals autismespectrumstoornis (ASS) en aandachttekort hyperactiviteit stoornis (ADHD). Dit impliceert dat SCT als belangrijk model kan dienen om ons begrip over werkingsmechanismen die bijdragen aan gedragsproblemen en psychopathologie te begrijpen.

Tot voor kort lag de focus van studies naar de gevolgen van SCT voornamelijk op lichamelijke en somatische consequenties van het extra chromosoom. Onderzoeken die zich hebben gericht op gedragsuitkomsten en het neurocognitieve profiel zijn zeldzamer. Met andere woorden, om de impact van SCT op de neurocognitieve ontwikkeling te begrijpen is het wenselijk dat meer studies zich richten op de neurocognitieve sterktes en zwaktes in deze populatie en op het verband van deze sterktes en zwaktes met uitkomsten op gedragsniveau. Het is om twee redenen belangrijk dat deze studies de ontwikkeling van kinderen in kaart brengen. Ten eerste omdat neurocognitieve functies vroeg in het leven voorlopers kunnen zijn van latere neurocognitieve en gedragsmatige uitkomsten, en zo kunnen dienen als signaal voor ontwikkelingsrisico. SCT kan prenataal vastgesteld worden, waardoor de vroege impact op ontwikkeling in kaart kan worden gebracht. Dit biedt de mogelijkheid om meer te leren over vroege ontwikkelingspaden die kunnen leiden tot psychopathologie. Ten tweede ontwikkelen (voorlopers van) neurocognitieve functies zich vroeg in het leven wanneer de ontwikkeling van het brein een snelle groei doormaakt. Hierdoor is het brein juist op jonge leeftijd ontvankelijk voor invloeden vanuit de omgeving, waarin factoren tijdens deze zogenaamde kritische perioden van snelle groei het ontwikkelingspad ingrijpend kunnen beïnvloeden.

Het doel van de studies die zijn opgenomen in dit proefschrift is om meer kennis over de vroege ontwikkeling van jonge kinderen met SCT te vergaren, zowel op het niveau van de gedragsexpressie als binnen een specifiek domein van informatieverwerking, namelijk taal en communicatie. Daarnaast is het doel van studies binnen dit proefschrift om prospectief de relatie tussen communicatie en gedrag te bestuderen. Met andere woorden: om bouwstenen van gedragsuitkomsten te identificeren. Alle onderzoeken die hebben geleid tot dit proefschrift werden afgenomen bij jonge kinderen in de leeftijd van 1-7 jaar.

Gezien het risico voor psychopathologie in de latere ontwikkeling van kinderen met SCT, is het van belang om naar de vroege presentatie van gedragsuitkomsten te kijken. De resultaten van dit proefschrift laten een hogere incidentie van gedragsproblemen bij jonge kinderen met SCT zien. Leeftijdsspecifieke gedragsprofielen illustreren daarbij dat sommige gedragsproblemen, zoals sociaal-emotionele problemen, al bij hele jonge kinderen van één jaar oud zichtbaar kunnen zijn. Andere gedragsproblemen laten een graduele toename met leeftijd zien, waarbij ook het ontwikkelingspad anders lijkt te zijn bij kinderen met SCT dan bij met leeftijdsgenoten; hierbij moet opgemerkt worden dat deze resultaten voortkomen uit cross-sectioneel onderzoek (**Hoofdstuk 2**). In dit proefschrift werden door middel van een review van de literatuur vier neurocognitieve domeinen geïdentificeerd als mogelijke bouwstenen van gedragsuitkomsten, namelijk het globaal intellectueel functioneren, sociale cognitie, executief functioneren, en taal en communicatie (**Hoofdstuk 3**). Taal vaak wordt omschreven als één van de meest kwetsbare neurocognitieve domeinen bij mensen met SCT. Daarnaast dienen taal- en communicatievaardigheden als belangrijke fundering voor vele andere neurocognitieve functies. Ook worden problemen met taal- en communicatie in andere populaties geassocieerd met nadelige gedragsuitkomsten en sociaal-emotionele ontwikkelingsproblemen. Om deze redenen ligt de focus van dit proefschrift op het taal- en communicatiedomein; een associatie die binnen de SCT populatie nog niet is onderzocht. De resultaten van **Hoofdstuk 4, 5, en 6,** illustreren dat taal en communicatieproblemen al op jonge leeftijd voor kunnen komen bij kinderen met SCT en dat meerdere communicatieve functies kwetsbaar zijn. Kwetsbaarheden in het taaldomein kunnen zich al voordoen vóór kinderen gesproken taal gebruiken om te communiceren (**Hoofdstuk 4**). Communicatieve problemen reiken verder dan structurele taalvaardigheden; sociale taal oftewel pragmatiek lijkt ook aangedaan te zijn (**Hoofdstuk 5**). Gedurende communicatieve interactie lijken ook sociale oriëntatie en het moduleren van interne *arousal* niet optimaal te zijn (**Hoofdstuk 6**). Daarnaast zijn taal- en communicatie voorspellers voor verschillende gedragsuitkomsten een jaar later (**Hoofdstuk 6**). Tot slot zijn sociale oriëntatie en het moduleren van *arousal* bij kinderen uit een populatiesteekproef gerelateerd aan sociaal gedrag in het dagelijks leven (**Hoofdstuk 7**). Samengenomen worden vroege communicatieve vaardigheden geassocieerd met gedragsuitkomsten. Deze bevindingen benadrukken het belang van vroege monitoring van deze vaardigheden, aangezien de vroege kindertijd een belangrijk moment kan zijn om de verdere ontwikkeling positief te beïnvloeden.

In de komende paragrafen worden de belangrijkste bevindingen van deze studies verder besproken. Vervolgens volgen de algemene discussie en worden implicaties van de bevindingen en suggesties voor toekomstig onderzoek besproken. Tot slot volgt er een conclusie van de belangrijkste bevindingen binnen dit proefschrift.

Samenvatting

Het Gedragsprofiel van Jonge Kinderen met SCT

Het doel van de studie zoals beschreven in **Hoofdstuk 2** was om het vroege gedragsprofiel van jonge kinderen met SCT te beschrijven en om vast te stellen of er sprake is van leeftijdsspecifieke profielen. De geïncludeerde kinderen tussen de 1 en 5 jaar met SCT lieten een hogere mate van sociaal-emotionele problemen, affectieve problemen, en pervasieve ontwikkelingsproblemen zien vergeleken met leeftijdsgenoten. Een klinische inschatting van de gedragsproblemen liet een hoge mate van variabiliteit zien binnen de SCT groep; sommige kinderen lieten weinig tot geen gedragsproblemen zien, terwijl andere kinderen in het klinisch gebied scoorden. In vergelijking met de controlegroep hadden kinderen met SCT vaker een

score die geïnterpreteerd wordt als 'verhoogd risico' of 'klinisch' voor de gedragsuitkomsten sociaal-emotionele problemen (40%), affectieve problemen (11%), angstproblemen (16%), en pervasieve ontwikkelingsproblemen (38%). Wanneer de groep werd opgesplitst in subgroepen op basis van leeftijd kwamen leeftijdsspecifieke profielen naar voren. In de groep van eenjarige kinderen met SCT liet een deel van de kinderen al sociaal-emotionele problemen zien. Dit type problematiek bleek persistent over de gehele leeftijdsrange van 1-5 jaar. Affectieve problemen en pervasieve ontwikkelingsproblemen waren zichtbaar bij kinderen vanaf 3 jaar oud, en prominenter bij 4-5 jarigen. Angstproblemen, aandachtsproblemen, en oppositioneel opstandig gedrag kwamen vaker voor bij 4-5 jarige kinderen met SCT vergeleken met leeftijdsgenoten. Daarnaast leek het ontwikkelingspad anders te zijn voor kinderen met SCT voor affectieve problemen, pervasieve ontwikkelingsproblemen, en oppositioneel opstandig gedrag. Sociaal-emotionele problemen daarentegen waren stabiel en persistent binnen de gehele leeftijdsrange van 1-5 jaar. Samengenomen lieten de resultaten zien dat kinderen met SCT al op jonge leeftijd een verhoogd risico hebben op suboptimale gedragsontwikkeling; een risico dat lijkt toe te nemen in ernst en uit te breiden naar verschillende soorten gedragingen met toenemende leeftijd.

Bouwstenen van Gedrag: Identificatie van Neurocognitieve Domeinen

Door middel van een *review* van de literatuur werd in **Hoofdstuk 3** gekeken naar mogelijke neurocognitieve bouwstenen die onderliggend zijn aan de kwetsbaarheden in het gedrag. Hiervoor werd bewijs van (neuro)cognitieve beperkingen in de domeinen globaal intellectueel functioneren, taalontwikkeling, executief functioneren, en sociale cognitie geëvalueerd. Het doel van deze literatuurreview was tweeledig. Ten eerste om de bestaande literatuur te bestuderen om zo eventuele gebreken te identificeren die vervolgens als uitgangspunt kunnen dienen voor toekomstig onderzoek. Ten tweede om te identificeren welke kwetsbaarheden in de neurocognitieve vaardigheden mogelijk kunnen dienen als uitgangspunt voor support of interventieprogramma's. Bij eerdere reviews lag de focus op ontwikkeling van individuen met SCT gedurende het hele leven, waarbij met name aandacht werd geschonken aan de adolescentie en volwassenheid. Binnen het huidige review lag de focus om deze reden op de vroege ontwikkeling.

De resultaten van deze review illustreren de noodzaak van onderzoek bij jonge kinderen, met name naar longitudinale studies waarbij het ontwikkelingspad over langere tijd gevolgd wordt. Binnen het domein globaal intellectueel functioneren was veel variatie in gerapporteerde uitkomsten, deze varieerden van een 'beperkt' globaal intellectueel functioneren tot een 'boven gemiddeld' globaal intellectueel functioneren. Over de acht geïncludeerde studies genomen, wordt het globaal intellectueel functioneren van kinderen met SCT geschat in een gemiddelde tot laag-gemiddelde range. Binnen het taaldomein worden kwetsbaarheden al op jonge leeftijd gerapporteerd; en deze kwetsbaarheden lijken persistent aan te houden gedurende de ontwikkeling. Over de vijf geïncludeerde studies genomen geeft de berekende effect grootte Cohen's *d* een hoge mate van klinische significantie aan (Cohen's $d > 1.00$), wat het belang van vroege detectie en support binnen het taaldomein illustreert. Binnen het domein van executief functioneren waren de uitkomsten van studies variabel, afhankelijk van de specifieke functie die werd bekeken. Over de vijf geïncludeerde studies samengenomen geeft de berekende effectgrootte een lage tot hoge mate van klinische significantie aan. Tot slot werd binnen het domein sociale cognitie over geïncludeerde studies samengenomen een gemiddelde tot hoge klinische significantie gevonden op basis van de berekende effectgrootte.

Concluderend kan gesteld worden dat de resultaten van deze review een duidelijk gebrek aan onderzoek naar de impact van SCT op de vroege ontwikkeling laten zien. Desalniettemin wijst de bestaande literatuur erop dat de aanwezigheid van het extra X of Y chromosoom impact heeft op het neurocognitief functioneren. Meer kennis van de vroege ontwikkeling op deze vier neurocognitieve domeinen kan leiden tot verbeterde klinische zorg met name door doelen voor vroege support en interventieprogramma's te identificeren.

Vroege Taal- en Communicatievaardigheden van Kinderen met SCT

Aangezien taal een kwetsbaar neurocognitief domein is bij individuen met SCT, taal- en communicatievaardigheden al vroeg in het leven ontwikkelen, en omdat taal en communicatie betrokken zijn bij de ontwikkeling van andere neurocognitieve functies, lag de focus van drie studies binnen dit proefschrift op de vroege taal- en communicatievaardigheden van kinderen met SCT. De studie in **Hoofdstuk 4** had als doel om de taalvermogens van kinderen met SCT in beeld te brengen in verschillende ontwikkelingsfases binnen de leeftijdsrange van 1-6 jaar en om de variabiliteit te beschrijven door middel van klinische classificaties van deze taalvermogens. Wat betreft de taalvermogens binnen verschillende ontwikkelingsfasen, lieten de resultaten zien dat kinderen van één jaar oud met SCT gemiddeld minder woorden produceren en begrijpen en over minder receptieve en expressieve semantische vaardigheden beschikken dan leeftijdsgenoten in de controlegroep. In onze onderzoeksgroep van kinderen van 3-4 jaar werd gevonden dat kinderen met SCT vergelijkbare receptieve semantische en receptieve syntactische taalvaardigheden hadden als leeftijdgenoten. De expressieve semantische taalvaardigheden van kinderen met SCT in deze leeftijdsgroep waren gemiddeld minder goed dan van leeftijdsgenoten. Tot slot vonden we mindere receptieve semantische, expressieve semantische, en receptieve syntactische taalvaardigheden bij kinderen van 5-6 jaar met SCT dan bij leeftijdsgenoten. Wat betreft de klinische classificaties lieten onze resultaten veel variabiliteit zien, waarbij de mate van klinisch relevante problemen – dat wil zeggen, het percentage kinderen dat onder een bepaalde score uitkwam – uiteenliep van 12 tot 50%, afhankelijk van het onderzochte taalaspect. Daarbij was het relatieve risico op taalproblemen, zoals berekend met de *odds ratio,* twee tot zeven keer zo hoog voor kinderen met SCT, wederom afhankelijk van het onderzochte taalaspect. In de studie in **Hoofdstuk 5** werd onderzocht hoe kinderen taal gebruiken in een sociale omgeving, ook wel pragmatiek genoemd. Onze resultaten lieten zien dat kinderen met SCT tussen de 3-7 jaar meer moeite hadden met alle drie de onderzochte aspecten van pragmatiek: (1) Non-verbale communicatie, (2) gespreksroutines en -vaardigheden, en (3) vragen om informatie, informatie geven en reageren op informatie. Kwetsbaarheden in pragmatiek waren niet beperkt tot kinderen die daarnaast ook structurele taalproblemen hadden, maar waren aanwezig over de volle breedte van de SCT groep. Het relatieve risico op inadequate pragmatische vaardigheden, zoals berekend met de *odds ratio,* lag 23 keer zo hoog in de SCT groep dan bij leeftijdsgenoten uit de controlegroep. Tot slot was het doel van de studie in **Hoofdstuk 6** om de kennis te verbreden hoe jonge kinderen reageren op korte periodes van communicatieve interactie, gemeten door middel van een dynamisch eye tracking paradigma en fysiologische arousal metingen. Onze resultaten lieten zien dat kinderen met SCT in de leeftijd van 1-7 jaar gemiddeld minderen oriënteren op sociale aspecten tijdens zulke communicatieve interacties, waarbij met name minder oriëntatie gevonden werd naar de ogen van de communicatieve partner op het computerscherm. Daarnaast lieten kinderen met SCT in reactie op verschillende situationele eisen, zoals een wisseling in de kijkrichting van de communicatieve partner, geen aanpassing zien in arousal levels. De resultaten van deze studie wijzen erop dat kinderen met SCT mogelijk moeite hebben met

sociale communicatie en dat de kwetsbaarheden in sociale communicatie verder reiken dan vertragingen in de vroege taalontwikkeling.

Samengenomen dragen de bovenstaande studies bij aan de toenemende literatuur over taal- en communicatieproblemen binnen de SCT populatie. Bovendien breiden deze studies de kennis uit door gericht onderzoek te doen naar jonge kinderen, specifieke taaluitkomsten, en door vaardigheden binnen het bredere domein van communicatie te bestuderen. Onze resultaten laten zien dat taal- en communicatieproblemen zich binnen verschillende ontwikkelingsfases voordoen en dat verscheidende vaardigheden binnen dit domein zijn aangedaan, waardoor het aannemelijk is dat deze problemen verankerd liggen in de vroege ontwikkeling van het brein.

Taal, Communicatie en Gedrag – Associaties tussen Huidige en Latere Vaardigheden

In drie studies hebben we associaties tussen taal, communicatie, en gedrag bekeken om te ontrafelen welke taal- en communicatievaardigheden mogelijk dienen als bouwstenen voor gedragsuitkomsten. In **Hoofdstuk 6** onderzochten we de relatie tussen sociale oriëntatie op het gezicht, de ogen, en de mond van een communicatieve partner op het computerscherm en receptieve en expressieve semantische vaardigheden gemeten op hetzelfde moment en één jaar later. Onze resultaten lieten positieve correlaties zien bij kinderen van één jaar met SCT: Kinderen die méér aandacht hadden voor belangrijke communicatieve onderdelen van het gezicht, dat wil zeggen de mond van de ander, hadden zowel op één jarige leeftijd als en een jaar later mínder taalproblemen. Deze resultaten illustreren dat sociale oriëntatie en taalvaardigheden sterk met elkaar verweven zijn op jonge leeftijd. De studie zoals beschreven in **Hoofdstuk 7** had als doel te evalueren in welke mate sociale gedragingen in het dagelijks leven gerelateerd zijn aan sociale oriëntatie en het aanpassen van het arousal niveau, zoals gemeten in reactie op sociale paradigma's, bij jonge kinderen uit een populatiesteekproef. De resultaten van deze studie illustreren dat sociale oriëntatie, met name aandacht voor ogen, gerelateerd is aan gedragingen in het dagelijks leven, zoals initiëren van sociale interactie, initiëren van gedragsverzoeken, sociaal bewustzijn, sociale cognitie, en het algeheel sociaal adaptief vermogen. Hierbij speelde ook de 'sociale lading' van de paradigma's een rol; het paradigma met de meeste sociale prikkels (een video van sociale interactie tussen personen) was gerelateerd aan meer gedragsuitkomsten in het dagelijks leven dan paradigma's met minder sociale prikkels (een video van één persoon of meerdere personen zonder interactie). Daarnaast werd een sterke fysiologische arousal respons geassocieerd met minder sociale oriëntatie op de ogen, en vervolgens met minder sociaal bewustzijn in het dagelijks leven. Tot slot illustreren de resultaten in **Hoofdstuk 5** de relevantie van structurele taal en pragmatiek voor gedragsuitkomsten één jaar later. Verminderde pragmatische en structurele taalvaardigheden waren voorspellend voor meer aandachtsproblemen, pervasieve ontwikkelingsproblemen, en sociaal-emotionele problemen één jaar later. Verminderde pragmatische taalvaardigheden waren daarnaast ook voorspellend voor meer affectieve problemen en oppositioneel opstandige gedragsproblemen. Met name pragmatische taalproblemen waren voorspellend voor een breed scala aan gedragsuitkomsten; sociaal-communicatieve vaardigheden kunnen mogelijk dienen als vroeg kenmerk voor latere gedragsproblemen en kunnen mogelijk ook een deel van de variantie in gedragsuitkomsten verklaren.

Samengenomen wijzen de resultaten van deze studies op kwetsbaarheden in het communicatiedomein van kinderen met SCT, die verder reiken dan structurele taalproblemen. Hieronder vallen verschillende vaardigheden die nodig zijn om op adequate wijze de sociale

wereld te begrijpen en erin te navigeren; communicatieve vaardigheden die lijken te dienen als bouwstenen voor latere gedragsuitkomsten.

Algemene Discussie

Het centrale doel van dit proefschrift was om kennis te vergaren over vroege taalvaardigheden van jonge kinderen met SCT binnen het bredere communicatiedomein en om prospectief de relatie tussen taal, communicatie, en gedrag te bestuderen. Op basis van de studies in dit proefschrift kunnen vier overkoepelende conclusies getrokken worden.

Conclusie 1: Moeilijkheden met Communicatie Reiken Verder dan Kwetsbaarheden in het Taaldomein

Taal is een kwetsbaar cognitief domein bij kinderen met SCT. Echter, kinderen met SCT kunnen problemen ervaren met communicatie die verder reiken dan structurele taalproblemen. In ons onderzoek kwam naar voren dat verschillende taal- en communicatieve functies betrokken kunnen zijn, waaronder functies die betrekking hebben op de vorm en inhoud van taal, het gebruik van taal als een sociaal hulpmiddel, en het vermogen om te vertrouwen op een sociaal 'kompas' wat nodig is om tijdens sociaal communicatieve interacties te kunnen navigeren. Zowel het begrip of de receptieve vermogens als de productie of expressieve vermogens kunnen aangedaan zijn. Taal speelt een belangrijke rol in de cognitieve en sociale ontwikkeling (Simms, 2007), en taal is een vereiste om te kunnen communiceren over behoeften, gedachten, en emoties. Taal en communicaties zijn ook nodig om te kunnen leren, te reflecteren op ervaringen en om de wereld om ons heen te kunnen begrijpen. Aangezien taal en communicatie nauw verweven zijn met verscheidene andere functies kunnen er ernstige consequenties zijn voor de ontwikkeling van andere neurocognitieve functies en gedragsuitkomsten wanneer zich problemen voordoen in de taal- en communicatievaardigheden. Dit kan vervolgens doorwerken met gevolgen voor het vermogen om deel te nemen aan de maatschappij of de ervaren kwaliteit van leven.

Conclusie 2: Taal- en Communicatievaardigheden als Bouwstenen voor Gedrag

Al van jongs af aan hebben kinderen met SCT een verhoogd risico op verschillende gedragsproblemen. Deze bevinding draagt bij aan de toenemende kennis over een verhoogd risico op ernstige gedragsproblemen bij SCT. Gedragsproblemen worden negatief geassocieerd met andere uitkomsten, waaronder het dagelijks functioneren, sociale competentie, schoolprestaties, en acceptatie van leeftijdsgenoten (de Lijster et al., 2019). Daarnaast kunnen gedragsproblemen in de kindertijd voorspellend zijn voor het risico op psychopathologie later in het leven (Goodwin et al., 2004; Ormel et al., 2015; Roza et al., 2003). Dit illustreert het belang om te ontrafelen welke mechanismen onderliggend zijn aan dit verhoogde risico en om signalen voor een 'risicovol' ontwikkelingspad op te sporen. Door deze signalen vroeg te signaleren en op basis hiervan de ontwikkeling zo optimaal mogelijk te stimuleren en ondersteunen, kan het risico op ernstigere psychopathologie later in het leven mogelijk verminderd worden. De studies in dit proefschrift laten zien dat taal en communicatie mogelijke neurocognitieve bouwstenen zijn die onderliggend zijn aan dit verhoogde risico en/of kunnen dienen als vroege signalen van dit verhoogde risico. Kwetsbare taal- en communicatieve vermogens kunnen voorafgaan aan of leiden tot verschillende nadelige gedragsuitkomsten; dit benadrukt het belang om te kijken naar preventieve ondersteuning en het belang te onderzoeken of het verbeteren van taal- en communicatievaardigheden mogelijk ook een positieve impact

kan hebben op de ontwikkeling van kinderen met mogelijk gunstigere gedragsuitkomsten tot gevolg.

Conclusie 3: Het Belang van het Ontwikkelingsperspectief

Om de impact van SCT op zowel neurocognitieve als gedragsuitkomsten te kunnen begrijpen is het belangrijk om het ontwikkelingsperspectief in acht te nemen. In de studies zoals gepresenteerd in **Hoofdstukken 2, 4, 5, en 6** werden jonge kinderen met SCT geïncludeerd om de kennis van de vroege ontwikkeling van deze groep te vergroten. De meerderheid van voorgaande studies heeft zich gericht op kinderen in de schoolleeftijd, adolescenten, en/of volwassenen. De studies uit dit proefschrift vullen daarmee een belangrijke lacune in de kennis. Naast dat meer kennis over de ontwikkeling en het ontwikkelingsrisico van taal en communicatie bij SCT de SCT populatie beter kan bedienen, kan deze kennis ook helpen om 'risicovolle' ontwikkelingspaden in de algehele populatie beter te begrijpen. In tegenstelling tot studies waarbij kinderen geïncludeerd worden die een diagnose hebben ontvangen op basis van een gedragsclassificatie, waarbij de ontwikkeling vaak alleen retrospectief in kaart gebracht kan worden, zorgt het bestuderen van een groep kinderen met een genetische diagnose voor de unieke mogelijkheid om prospectief vroege markers en ontwikkelingspaden van een risicovolle ontwikkeling te bestuderen, zoals bijvoorbeeld in de meest vroege fases van communicatieve ontwikkeling.

Om onze kennis van de vroege ontwikkeling van kinderen met SCT te vergroten, werd bij alle studies in dit proefschrift leeftijd binnen de range van 1-7 jaar meegenomen om de factor ontwikkeling mee te kunnen nemen bij de interpretatie van de resultaten. Hierbij werd specifiek gekeken naar uitkomsten binnen een bepaald leeftijdsbereik of ontwikkelingspaden werden in beeld gebracht op basis van cross-sectionele data. Resultaten van **Hoofdstukken 2, 4, 5, en 6** illustreren dat kinderen met SCT een verhoogd risico hebben op sociaal-emotionele problemen, al vanaf de leeftijd van één jaar. Daarnaast kunnen zich op deze leeftijd problemen in het taal- en communicatiedomein voordoen, waaronder het begrijpen en gebruiken van minder woorden, het ervaren van moeilijkheden met andere semantische vaardigheden, en het minder gebruiken van vaardigheden zoals sociale oriëntatie en het aanpassen van fysiologische activatie (arousal) in reactie op een sociale situatie. Vanaf de leeftijd van 3-4 jaar komen affectieve en pervasieve ontwikkelingsproblemen meer naar voren, naast de ervaren (globale) sociaal-emotionele problemen. In het taal- en communicatiedomein kunnen kinderen meer problemen ervaren met expressieve semantiek (de productie van woorden en zinnen), met pragmatiek (het gebruik van taal in een sociale context), en in het gebruik van vaardigheden tijdens sociale interactie (sociale interactie en aanpassen van arousal). Tot slot kan bij kinderen met SCT vanaf 5-7 jaar een verhoogd risico ontstaan voor sociaal-emotionele problemen, affectieve problemen, angst, pervasieve ontwikkelingsproblemen, aandachtsproblemen, en oppositioneel opstandig gedrag. In het taal- en communicatiedomein kunnen zowel receptieve als expressieve semantiek, syntactische vaardigheden (combineren van woorden tot zinnen en grammatica), en pragmatiek aangedaan zijn, naast de sociale oriëntatie en de modulatie van arousal tijdens sociale interactie.

Onze bevindingen illustreren dat taal- en communicatievermogens al vanaf jonge leeftijd aangedaan kunnen zijn bij kinderen met SCT. Dit sluit aan bij resultaten van *neuroimaging* onderzoeken, op basis waarvan wordt geconcludeerd dat de anatomie en het functioneren van het brein aangedaan kunnen zijn door de aanwezigheid van een extra X of Y chromosoom (Brandenburg-Goddard et al., 2014; Bryant et al., 2012; Giedd et al., 2007; Lenroot et al., 2014; Lentini et al., 2013; Nadig et al., 2018; Patwardhan et al., 2002; Raznahan

et al., 2016; van Rijn et al., 2008; van Rijn et al., 2012; Warling et al., 2020). De impact van SCT op de vroege taal- en communicatievermogens in combinatie met de bevindingen van deze *neuroimaging* onderzoeken passen bij de gedachte dat de kwetsbaarheden binnen dit domein verankerd zijn in de vroege ontwikkeling van het brein. De eerste levensjaren worden gekenmerkt door een vlotte ontwikkeling van het brein. Zo wordt het volume van het brein gedurende deze periode meer dan verdubbeld en is er een enorme toename van structurele en functionele netwerken. Hoewel de rijping van het brein zich na deze eerste levensjaren voortzet, ligt het tempo waarop dit gebeurd aanzienlijk lager dan in de vroege kindertijd (Gilmore et al., 2018). Door deze vlotte ontwikkeling is het brein in de eerste levensjaren meer vatbaar voor verandering. Vroeg ingezette interventies hebben daarom mogelijk meer impact op de suboptimale ontwikkeling van het brein dan interventies die later in de ontwikkeling worden ingezet. Aangezien het risico voor suboptimale ontwikkeling al vroeg in het leven aanwezig is bij kinderen met SCT én aangezien dit risico lijkt toe te nemen en uit te breiden wanneer kinderen ouder worden, wordt het belang van vroege monitoring en interventies waarmee het ontwikkelingspad van kinderen met SCT mogelijk positief beïnvloed kan worden benadrukt.

Conclusie 4: Robuuste Kwetsbaarheden

Binnen onze studies werd geen bewijs gevonden dat kenmerken van SCT, zoals het specifieke karyotype, tijd van diagnose, of de reden van deelname aan het onderzoek, een significante rol spelen in het verklaren van de uitkomsten. In **Hoofdstukken 2, 4, 5, en 6** werd verkend of deze kenmerken een impact hadden op de gevonden resultaten. Wat betreft SCT karyotypes werden enkele verschillen gevonden in het gedragsprofiel wanneer kinderen in de SCT groep vergeleken werden met leeftijdsgenoten van hetzelfde geslacht (XXX versus XX, XXY versus XY, XYY versus XY). Affectieve en sociaal-emotionele problemen daarentegen kwamen bij alle drie de vormen van SCT voor. Onze resultaten gaven geen aanwijzing voor substantiële verschillen in de taal- en communicatievermogens van meisjes met een extra X, jongens met een extra X, of jongens met een extra Y. Wat betreft tijd van de diagnose vonden we dat kinderen met een postnatale diagnose meer (verschillende) gedragsproblemen lieten zien dan kinderen met een prenatale diagnose. Dit was verwacht, aangezien gedragsproblemen een aanleiding kunnen zijn voor genetisch onderzoek waarna een postnatale diagnose van SCT volgt. Sociaal-emotionele problemen daarentegen kwamen ook bij kinderen met een prenatale diagnose voor. Onze resultaten gaven geen aanwijzing van verschillen in de taal- en communicatievermogens van kinderen met een prenatale diagnose versus kinderen met een postnatale diagnose. Tot slot vonden we geen verschillen in taal, communicatie, of gedragsuitkomsten tussen kinderen die deelnamen aan de studie met als reden dat zijn prospectief gevolgd werden na prenatale diagnose, omdat ouders informatie zochten, of nadat zij vanuit klinische behandeling doorverwezen werden naar het onderzoek. Samengenomen lijkt de aanwezigheid van een extra X of Y chromosoom een grotere impact te hebben op taal, communicatie, sociaal-emotionele en gedragsuitkomsten dan specifieke kenmerken van SCT zoals het karyotype, tijd van diagnose, of de reden van deelname; de kwetsbaarheden die werden geïdentificeerd in onze studies lijken robuust te zijn binnen de SCT groep.

Klinische Implicaties

De resultaten van dit proefschrift illustreren dat – als groep – kinderen met SCT een verhoogd risico lopen op zowel nadelige gedragsuitkomsten, als kwetsbaarheden binnen het taal- en communicatie domein. De resultaten van onze studies wijzen erop dat er een hoge mate van variabiliteit is binnen de SCT groep, waarbij sommige kinderen ernstige problemen ervaren,

terwijl andere kinderen geen of nauwelijks problemen ervaren en niet merkbaar verschillen in cognitief en sociaal-emotioneel functioneren van leeftijdgenoten. Het is belangrijk op te merken dat de resultaten zoals gepresenteerd in dit proefschrift het gemiddelde van de geïncludeerde groep kinderen met SCT reflecteren, terwijl ieder kind uniek is en ieder kind op zijn of haar eigen tempo ontwikkelt. De bevindingen zoals gerapporteerd in dit proefschrift hebben drie klinische implicaties.

Implicatie 1: Belang van Vroege Monitoring en Identificatie

We benadrukken het belang om naast taalvaardigheden ook vaardigheden in het bredere communicatie domein op te nemen in de screening van ontwikkelingsrisico's bij SCT op jonge leeftijd om het mogelijke risico op nadelige gedragsuitkomsten vroeg te identificeren. Wanneer een kind de vastgestelde mijlpalen voor zijn of haar leeftijd niet tijdig bereikt, wordt neuropsychologisch onderzoek geadviseerd in plaats van het beloop van de ontwikkeling af te wachten. Bij deze screening op ontwikkelingsrisico's lijkt het aanbevolen ook de vroege non-verbale communicatie en sociale aspecten van communicatie op te nemen. Op basis van de bevinding dat gedragsproblemen toe lijken te nemen bij oudere kinderen in het leeftijdsdomein van 1-7 jaar, wordt aanbevolen het ontwikkelingspad van taal- en communicatievaardigheden nauwlettend te volgen.

Implicatie 2: Belang van Vroege Preventieve Support of Interventie

Het ontwikkelen van communicatie- en taalvaardigheden is een belangrijke taak voor jonge kinderen en moeilijkheden in het verkrijgen van deze vaardigheden kunnen impact hebben op uitkomsten op andere ontwikkelingsdomeinen. Op basis van resultaten uit de studies in dit proefschrift wordt sociale communicatie als belangrijke bouwsteen voor verschillende gedragsuitkomsten geïdentificeerd. Daarnaast werd bij heel jonge kinderen gevonden dat taalvermogens (expressieve en receptieve semantiek) sterk samenhangen met sociale oriëntatie, zowel wanneer deze uitkomsten gelijktijdig in kaart gebracht werden als wanneer gekeken werd naar taalvermogens een jaar later. Deze bevindingen benadrukken het belang van het verstrekken van preventieve support en/of vroege interventies. De vroege ontwikkeling is een belangrijke fase waarbij effectieve interventie cruciaal kan zijn om positieve sociale en academische uitkomsten later in het leven veilig te stellen (Kaiser & Roberts, 2011). Het is belangrijk om de effectiviteit van bestaande wetenschappelijk bewezen support en interventie programma's te evalueren voor kinderen met SCT en – als deze programma's niet voldoen – op maat gemaakte programma's te ontwikkelen specifiek voor de behoeftes van kinderen met SCT.

Implicatie 3: Belang van Actuele Informatie voor Professionals en Ouders

In het domein van de zeldzame genetische syndromen is het belangrijk dat professionals de mogelijke impact van SCT op de ontwikkeling kennen. De rol van 'expert' moet niet op de schouders van de ouders rusten (Richardson et al., 2021). Binnen de multidisciplinaire teams die zorg verlenen bij SCT is het belangrijk dat professionals, waaronder klinisch genetici, kinderartsen, ontwikkelingspsychologen, logopedisten, en fysiotherapeuten op de hoogte zijn en blijven van kennis over de impact van genetische aandoeningen zoals SCT. Zo kunnen professionals ervoor zorgen dat specifieke domeinen nauwlettend in de gaten gehouden kunnen worden tijdens kwetsbare ontwikkelingsfases.

Op basis van onze bevindingen hechten wij er belang aan dat professionals bewust zijn van de grote variabiliteit in (ontwikkelings-)uitkomsten van SCT. Professionals kunnen met

deze kennis ouders op de hoogte stellen van deze grote variabiliteit in uitkomsten wanneer ouders de diagnose ontvangen. Ouders kunnen waardevolle informatie verstrekken aan professionals, zorgen van ouders zijn vaak belangrijke markers voor vroege opsporing van neurocognitieve- en gedragsproblemen (Glascoe & Dworking, 1995).

Professionals kunnen ouders informeren over mogelijkheden om de vroege ontwikkeling te ondersteunen. Om de taalontwikkeling van kinderen te stimuleren kan ouders bijvoorbeeld worden aangeraden om boeken te lezen met hun kind. Dit is een algemene aanbeveling die voor ieder kind belangrijk is, maar mogelijk cruciaal voor kinderen met een verhoogde kwetsbaarheid op taalproblematiek, zoals kinderen met SCT. Op basis van individuele neurocognitieve screening kan er zicht ontstaan op de *individuele* behoeften van het kind, waardoor ouders zich gesteund kunnen voelen in het bieden van een veilige en sensitieve omgeving waarin hun kind zich optimaal kan ontwikkelen.

Sterktes en Limitaties

Taal- en communicatievaardigheden zijn een kwetsbaar domein voor kinderen met SCT. Vaardigheden binnen dit domein kunnen belangrijk zijn om 'risicovolle' ontwikkelingspaden te identificeren en kunnen, in ieder geval gedeeltelijk, gedragsuitkomsten verklaren. Helaas zijn studies die de neurocognitieve en gedragsuitkomsten in SCT – met name bij jonge kinderen – hebben onderzocht zeldzaam. De studies binnen dit proefschrift zijn ontworpen om kennis op de domeinen van taal en communicatie bij SCT te vergroten, in de leeftijdsrange van 1-7 jaar.

Een sterke kant van ons onderzoek is de grote internationale steekproef van jonge kinderen met SCT. Door de grootte van de steekproef kon worden gekeken naar leeftijdsspecifieke uitkomsten binnen de range van 1-7 jaar en naar de impact van SCT kenmerken zoals karyotype, tijd van diagnose, en reden van deelname aan onderzoek. Doordat gedragsuitkomsten één jaar later nogmaals in kaart werden gebracht, kon ook gekeken worden naar voorspellende waarde van neurocognitieve uitkomsten op gedrag één jaar later.

Een limitatie van ons onderzoek was de vergelijkbaarheid tussen leeftijdsgroepen binnen de leeftijdsrange van 1-7 jaar. Meetinstrumenten voor kinderen van één jaar oud waren in veel gevallen anders dan de meetinstrumenten die werden gebruikt voor dezelfde concepten bij kinderen van drie tot zeven jaar oud. Om te voorkomen dat kinderen wisselden tussen meetinstrumenten na een jaar, werden tweejarige kinderen niet geïncludeerd. Daarnaast waren enkele meetinstrumenten slechts voor bepaalde leeftijden geschikt, waardoor kinderen van andere leeftijden geëxcludeerd werden voor bepaalde analyses. Tot slot leidt 'wervingsbias' veelal tot variantie in het SCT fenotype, waarbij sommige problemen mogelijk worden overschat, terwijl andere problemen worden onderschat. Door kinderen te includeren met zowel een prenatale als postnatale diagnose en ongeacht de reden voor deelname aan het onderzoek werd gepoogd deze 'bias' te beperken. Echter, aangezien niet alle families met een kind met SCT ervoor kiezen om deel te nemen aan wetenschappelijk onderzoek en aangezien genetisch onderzoek niet bij iedereen wordt uitgevoerd, kan niet worden uitgesloten dat de beschreven uitkomsten niet volledig representatief zijn voor de totale populatie kinderen met SCT.

Aanbevelingen voor Vervolgonderzoek

Op basis van de bevindingen van de studies in dit proefschrift komen enkele aanbevelingen voor vervolgonderzoek naar voren.

Allereerst, aangezien taal en communicatie vlot ontwikkelen in de eerste levensjaren wordt aanbevolen om het leeftijdsbereik uit te breiden in toekomstige studies, en om uitkomsten over een langere periode te volgen. Aangezien kinderen met SCT prenataal gediagnosticeerd kunnen worden zou het volgen van deze kinderen bij voorkeur al zo snel mogelijk na de geboorte starten, om zo meer kennis te vergaren over de hele vroege communicatieve ontwikkeling. Door de ontwikkeling van neurocognitieve functies over een langere tijd te bestuderen en door uitkomsten over tijd te voorspellen kan het inzicht in verschillende ontwikkelingspaden en factoren die deze paden sturen of beïnvloeden vergroot worden.

Ten tweede is meer kennis nodig over andere aspecten van de neurocognitieve ontwikkeling van kinderen met SCT waarvan bekend is dat de voorlopers zich al op jonge leeftijd manifesteren. Daarbij kan ook gekeken worden hoe deze neurocognitieve functies samenhangen met gedragsuitkomsten. Hieronder vallen niet alleen functies binnen het taal- en communicatiedomein; op basis van kwetsbaarheden die zijn geïdentificeerd bij oudere individuen met SCT is meer kennis over de vroege sociaal-cognitieve functies en executieve functies van jonge kinderen met SCT ook wenselijk. Er zijn tot nu toe weinig studies die onderzoeken hoe deze neurocognitieve functies samenhangen met gedragsuitkomsten of met elkaar op deze jonge leeftijd. Het is mogelijk dat kwetsbaarheden in neurocognitieve functies en gedragsproblemen samenhangen met problemen in metacognitieve controlefuncties. Een voorbeeld van een metacognitieve controlefunctie is zelfgestuurde spraak. Zelfgestuurde spraak ontwikkelt vanaf de peuterjaren; peuters praten hardop en openlijk tegen zichzelf. Geleidelijk aan ontwikkelt zich dit tot meer 'verborgen' spraak, bijvoorbeeld in de vorm van fluisteren tegen zichzelf, met name bij het uitvoeren van een moeilijke taak. Tot slot neemt zelfgestuurde spraak de vorm aan van innerlijke spraak (Mulvihill et al., 2020). Studies hebben laten zien dat minder goed ontwikkelde zelfgestuurde spraak kan resulteren in significante cognitieve en gedragsmoeilijkheden (Alderson-Day & Fernyhough, 2015; Whitehouse et al., 2006). Studies bij kinderen met ontwikkelingsstoornissen zoals een taalontwikkelingsstoornis, autismespectrumstoornis, of ADHD laten atypische ontwikkeling van zelfgestuurde spraak zien (voor een overzicht zie Mulvihill et al., 2020). Ook bij SCT zouden problemen in de ontwikkeling van zelfgestuurde spraak mogelijk samen kunnen hangen met de verhoogde prevalentie van ontwikkelingsstoornissen en een verhoogd risico op neurocognitieve problemen. Het kan daarom interessant zijn om het concept van zelfgestuurde spraak bij mensen met SCT te onderzoeken.

Ten derde is de ontwikkeling van kinderen dynamisch; er is een complexe relatie tussen omgevings- en interpersoonlijke factoren, neurocognitief functioneren, en gedragsuitkomsten. Meer kennis is nodig over de impact van omgevings- en interpersoonlijke factoren. Zo kan bijvoorbeeld de 'taalrijkheid' van de omgeving een risico- of bevorderende factor in de ontwikkeling van taal en communicatie zijn. Interpersoonlijke factoren, zoals bijvoorbeeld de ontvangen support van professionals en de leeftijd waarop het kind de support ontvangt kunnen ook impact hebben op latere uitkomsten. Hieronder valt ook bijvoorbeeld het effect van testosteronbehandeling. De effecten van deze omgevings- en interpersoonlijke factoren op neurocognitieve en gedragsuitkomsten kan verder worden verkend om zo meer zicht te krijgen op individuele ontwikkelingspaden en om risico- en bevorderende factoren te identificeren.

Tot slot verdient het aanbeveling bestaande interventieprogramma's te evalueren met betrekking tot inzetbaarheid voor kinderen met SCT. Resultaten van de studies in dit proefschrift laten zien dat het ondersteunen van sociaal communicatieve vaardigheden

belangrijk kan zijn om kinderen te beschermen voor het risico op ongunstige uitkomsten, zoals psychopathologie later in het leven. Interventies die ingezet worden bij andere populaties, bijvoorbeeld bij kinderen met een autismespectrumstoornis of een taalontwikkelingsstoornis en die zich richten op deze specifieke neurocognitieve bouwsteen kunnen mogelijk ook van belang zijn voor kinderen met SCT. Als bestaande interventieprogramma's niet toereikend zijn of niet bestaan, zouden specifieke interventies ontwikkeld kunnen worden die zich richten op de behoeften van kinderen met SCT.

Conclusies

De studies in dit proefschrift laten zien dat kinderen met SCT al vanaf zeer jonge leeftijd een verhoogde kwetsbaarheid hebben op nadelige gedragsuitkomsten en een verhoogd risico op neurocognitieve problemen in het taal- en communicatie domein. Dit risico voor taal- en communicatieproblemen en de kwetsbaarheid voor nadelige gedragsuitkomsten kan toenemen wanneer kinderen ouder worden. Onderzoek naar neurocognitieve functies op jonge leeftijd kan helpen vroege markers van risicovolle ontwikkelingspaden te onderkennen. Op basis van de bevindingen in onze studies lijkt vroege monitoring met daaraan gekoppelde programma's voor vroege support en interventie aan te bevelen. Samengenomen kan worden geconcludeerd dat het bestuderen van onderliggende mechanismen van nadelige uitkomsten via een *neurogenetische benadering* kan zorgen voor meer kennis van relaties tussen het brein en gedrag in het algemeen.

References

Alderson-Day, B., & Fernyhough, C. (2015). Inner Speech: Development, Cognitive Functions, Phenomenology, and Neurobiology. *Psychological Bulletin Journal*, *141*(5), 931-965. https://doi.org/10.1037/bul0000021

Brandenburg-Goddard, M. N., Van Rijn, S., Rombouts, S., Veer, I. M., & Swaab, H. (2014). A comparison of neural correlates underlying social cognition in Klinefelter syndrome and autism. *Social Cognitive and Affective Neuroscience*, *9*(12), 1926-1933. https://doi.org/10.1093/scan/nst190

Bryant, D. M., Hoeft, F., Lai, S., Lackey, J., Roeltgen, D., Ross, J., & Reiss, A. L. (2012). Sex chromosomes and the brain: A study of neuroanatomy in XYY syndrome. *Developmental Medicine and Child Neurology*, *54*(12), 1149-1156. https://doi.org/10.1111/j.1469-8749.2012.04418.x

de Lijster, J. M., van den Dries, M. A., van der Ende, J., Utens, E., Jaddoe, V. W., Dieleman, G. C., . . . Legerstee, J. S. (2019). Developmental trajectories of anxiety and depression symptoms from early to middle childhood: A population-based cohort study in the Netherlands. *Journal of Abnormal Child Psychology*, *47*(11), 1785-1798. https://doi.org/10.1007/s10802-019-00550-5

Giedd, J. N., Clasen, L. S., Wallace, G. L., Lenroot, R. K., Lerch, J. P., Wells, E. M., . . . Samango-Sprouse, C. A. (2007). XXY (Klinefelter syndrome): A pediatric quantitative brain magnetic resonance imaging case-control study. *Pediatrics*, *119*(1), e232-240. https://doi.org/10.1542/peds.2005-2969

Gilmore, J. H., Knickmeyer, R., & Gao, W. (2018). Imaging structural and functional brain development in early childhood. *Nature Reviews Neuroscience*, *19*(3), 123-137. https://doi.org/10.1038/nrn.2018.1

Glascoe, F. P., & Dworking, P. H. (1995). The role of parents in the detection of developmental and behavioral problems. *Pediatrics*, *95*(6), 829-836.

Goodwin, R. D., Fergusson, D. M., & Horwood, L. J. (2004). Early anxious/withdrawn behaviours predict later internalising disorders. *Journal of Child Psychology and Psychiatry*, *45*(4), 874-883. https://doi.org/10.1111/j.1469-7610.2004.00279.x

Kaiser, A. P., & Roberts, M. Y. (2011). Advances in early communication and language intervention. *Journal of Early Intervention*, *33*(4), 298-309. https://doi.org/10.1177/1053815111429968

Lenroot, R. K., Blumenthal, J. D., Wallace, G. L., Clasen, L. S., Lee, N. R., & Giedd, J. N. (2014). A case-control study of brain structure and behavioral characteristics in 47,XXX syndrome. *Genes Brain and Behavior*, *13*(8), 841-849. https://doi.org/10.1111/gbb.12180

Lentini, E., Kasahara, M., Arver, S., & Savic, I. (2013). Sex differences in the human brain and the impact of sex chromosomes and sex hormones. *Cerebral Cortex*, *23*(10), 2322-2336. https://doi.org/10.1093/cercor/bhs222

Mulvihill, A., Carroll, A., Dux, P. E., & Matthews, N. (2020). Self-directed speech and self-regulation in childhood neurodevelopmental disorders: Current findings and future directions. *Development and Psychopathology*, *32*(1), 205-217. https://doi.org/10.1017/S0954579418001670

Nadig, A., Reardon, P. K., Seidlitz, J., McDermott, C. L., Blumenthal, J. D., Clasen, L. S., . . . Raznahan, A. (2018). Carriage of supernumerary sex chromosomes decreases the volume and alters the shape of limbic structures. *eNeuro*, *5*(5). https://doi.org/10.1523/ENEURO.0265-18.2018

Ormel, J., Raven, D., van Oort, F., Hartman, C. A., Reijneveld, S. A., Veenstra, R., . . . Oldehinkel, A. J. (2015). Mental health in Dutch adolescents: A TRAILS report on prevalence, severity, age of onset, continuity and co-morbidity of DSM disorders. *Psychological Medicine*, *45*(2), 345-360. https://doi.org/10.1017/S0033291714001469

Patwardhan, A. J., Brown, W. E., Bender, B. G., Linden, M. G., Eliez, S., & Reiss, A. L. (2002). Reduced size of the amygdala in individuals with 47,XXY and 47,XXX karyotypes. *American Journal of Medical Genetics (Neuropsychiatric Genetics)*, *114*(1), 93-98. https://doi.org/10.1002/ajmg.10154

Raznahan, A., Lee, N. R., Greenstein, D., Wallace, G. L., Blumenthal, J. D., Clasen, L. S., & Giedd, J. N. (2016). Globally divergent but locally convergent X- and Y-chromosome influences on cortical development. *Cerebral Cortex*, *26*, 70-79. https://doi.org/10.1093/cercor/bhu174

Richardson, J. P., Riggan, K. A., & Allyse, M. (2021). The expert in the room: Parental advocacy for children with sex chromosome aneuploidies. *Journal of Developmental and Behavioral Pediatrics*, *42*(3), 213-219. https://doi.org/10.1097/DBP.0000000000000885

Roza, S. J., Hofstra, M. B., van der Ende, J., & Verhulst, F. C. (2003). Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: A 14-year follow-up during childhood, adolescence, and young adulthood. *American Journal of Psychiatry*, *160*, 2116-2121. https://doi.org/10.1176/appi.ajp.160.12.2116

Simms, M. D. (2007). Language disorders in children: Classification and clinical syndromes. *Pediatric Clinics of North America*, *54*, 437-467. https://doi.org/10.1016/j.pcl.2007.02.014

Van Rijn, S., Aleman, A., Swaab, H., Vink, M., Sommer, I., & Kahn, R. S. (2008). Effects of an extra X chromosome on language lateralization: An fMRI study with Klinefelter men (47,XXY). *Schizophrenia Research*, *101*(1-3), 17-25. https://doi.org/10.1016/j.schres.2008.02.001

Van Rijn, S., Swaab, H., Baas, D., de Haan, E., Kahn, R. S., & Aleman, A. (2012). Neural systems for social cognition in Klinefelter syndrome (47,XXY): Evidence from fMRI. *Social Cognitive and Affective Neuroscience*, *7*(6), 689-697. https://doi.org/10.1093/scan/nsr041

Warling, A., Liu, S., Wilson, K., Whitman, E., Lalonde, F. M., Clasen, L. S., . . . Raznahan, A. (2020). Sex chromosome aneuploidy alters the relationship between neuroanatomy and cognition. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *184*(2), 493-505. https://doi.org/10.1002/ajmg.c.31795

Whitehouse, A. J. O., Maybery, M. T., & Durkin, K. (2006). Inner speech impairments in autism. *Journal of Child Psychology and Psychiatry*, *47*(8), 857-865. https://doi.org/10.1111/j.1469-7610.2006.01624.x

Curriculum Vitae

Evelien Urbanus werd geboren op 27 november 1991 te Zoetermeer. Na het behalen van haar VWO diploma aan het Erasmus College te Zoetermeer in 2010, startte zij met de bachelor Psychologie aan de Universiteit Leiden. In 2013 volgde zij een uitwisselingsprogramma met Rutgers, the State University of New Jersey in de Verenigde Staten, waar zij haar bacheloropleiding heeft afgerond. Aansluitend aan het behalen van haar bacheloropleiding startte zij in Leiden met de tweejarige Research Master 'Developmental Psychology'. Tijdens deze Research Master heeft Evelien haar onderzoeksstage afgerond bij de afdeling Orthopedagogiek. Na het behalen van haar Research Master (*cum laude*) heeft Evelien gewerkt als onderzoeksmedewerker en docent bij de programmagroep Neuropedagogiek en Ontwikkelingsstoornissen aan de Universiteit Leiden. In 2019 werd Evelien aangesteld als promovendus onder begeleiding van dr. Sophie van Rijn en prof.dr. Hanna Swaab. Tijdens haar promotietraject heeft Evelien onderzoek gedaan naar de neurocognitieve vaardigheden en gedragsuitkomsten van jonge kinderen met een extra X of Y chromosoom. De resultaten van haar onderzoek staan beschreven in dit proefschrift. Naast haar promotietraject zette Evelien haar werkzaamheden als docent in de bachelor Pedagogische Wetenschappen en master Orthopedagogiek voort en behaalde zij haar Basiskwalificatie Onderwijs (BKO) en Basiskwalificatie Engels (BKE). Sinds oktober 2021 is Evelien werkzaam bij de programmagroep Klinische Neuropsychologie aan de Vrije Universiteit Amsterdam.

List of Publications

Journals

Urbanus, E., Swaab, H., Tartaglia, N., Boada, R., & Van Rijn, S. (2022). A cross-sectional study of early language abilities in children with sex chromosome trisomy (XXY, XXX, XYY) aged 1-6 years. *Child Neuropsychology, 28*(2), 171-196. https://doi/org/10.1080/09297049.2021.1960959

Urbanus, E. Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2020). The behavioral profile of children aged 1-5 years with sex chromosome trisomy (47,XXX, 47,XXY, 47, XYY). *American Journal of Medical Genetics Part C, 184C,* 444-455. https://doi.org/10.1002/ajmg.c.31788

Urbanus, E., Swaab, H. Tartaglia, N., Stumpel, C., & Van Rijn, S. (2022). Structural and pragmatic language in young children with sex chromosome trisomy (XXX, XXY, XYY): Predictive value for neurobehavioral problems one year later. *The Clinical Neuropsychologist – accepted manuscript.* https://doi.org/10.1080/13854046.2022.2067078

Urbanus, E., Swaab, H., Tartaglia, N., & Van Rijn, S. Social communication deficit in children with sex chromosome trisomy (XXY, XXX, XYY)? Social orientation measured with eye tracking and physiological arousal responses during short communicative interaction paradigms. *Submitted*.

Urbanus, E., Van Rijn, S., & Swaab, H. (2019). A review of neurocognitive functioning of children with sex chromosome trisomies: Identifying targets for early intervention. *Clinical Genetics, 97*(1), 156-167. https://doi.org/10.1111/cge.13586

Van Rijn, S., **Urbanus, E.**, & Swaab, H. (2018). Eyetracking measures of social attention in young children: How gaze patterns translate to real-life social behaviors. *Social Development*, *28*(3), 564-580. https://doi.org/10.1111/sode.12350

Abstracts

Urbanus, E., Swaab, H., Tartaglia, N., & Van Rijn, S. (June 21-27, 2021). *The behavioral profile of children with sex chromosome trisomy: Neurocognitive underpinnings of behavioral outcomes.* AXYS 2021 Virtual Family Conference, online conference.

Urbanus, E., Swaab, H., Tartaglia, N., & Van Rijn, S. (September 9-10, 2021). *The behavioral profile of children with sex chromosome trisomy: Neurocognitive underpinnings of behavioral outcomes.* Society for the Study of Behavioural Phenotypes (SSBP), online conference.

Urbanus, E., Swaab, H., Tartaglia, N., & Van Rijn, S. (2022). *Sociale communicatie bij kinderen met een extra X of Y chromosoom. Onderzoek met eye tracking en fysiologische arousal.* Nederlandse Vereniging voor Neuropsychologie (NVN), Nijmegen, the Netherlands.

Dankwoord

Toen ik tijdens mijn research master moest bedenken waar ik als onderdeel van mijn onderzoeksstage onderzoek naar wilde doen was de doelgroep al snel duidelijk; ontwikkelingsstoornissen, en met name de ontwikkeling van kinderen met genetische aandoeningen vond ik fascinerend. Wáár ik dat onderzoek kon gaan doen was nog wel een vraag, want binnen de afdeling waar ik mijn master volgde werd daar geen onderzoek naar gedaan. Na een zoektochtje kwam ik uit bij het project 'Neuropsychologische studies bij kinderen met genetische risico's' van Sophie van Rijn. Na een kennismaking in 2014(!) kon ik in het najaar als stagiaire starten op een nieuw project 'Growing up with sex chromosome trisomy: The social attention hypothesis' (bij de meesten bekend als 'de Pilot studie'), wat later de basis zou vormen voor de 'TRIXY early childhood study'. Dat die zoektocht naar een stageplek jaren geleden uiteindelijk zou uitmonden in een promotie had ik toen nog niet kunnen voorspellen. Uiteraard was dat niet gelukt zonder hulp, en hieronder wil ik graag een aantal mensen in het bijzonder bedanken.

Allereerst veel dank aan mijn promotoren prof.dr. Hanna Swaab en dr. Sophie van Rijn voor deze mogelijkheid en het vertrouwen dat ik in korte tijd toch een heel promotietraject zou kunnen doorlopen. Hanna, dank voor jouw kritische blik en jouw oneindige (klinische) kennis; ondanks dat ik zelf weinig praktijkervaring heb, hebben we de onderzoeksresultaten mooi kunnen verwoorden naar praktische handvatten voor de klinische praktijk. Sophie, veel dank dat ik destijds (is het al 8 jaar?!) bij jou mocht starten en dat ik na mijn afstuderen ook op jouw netvlies bleef, wat resulteerde in een mooie start van mijn loopbaan. Ik heb veel van je geleerd in de afgelopen jaren, en zal hier de rest van mijn loopbaan veel profijt van hebben.

Daarnaast wil ik mijn medepromovenda op het TRIXY project: Nienke Bouw en Kimberly Kuiper bedanken. Ik kijk met veel plezier terug op onze tijd samen en wat hebben we een hoop beleefd. Van 'gewoon' werken op kantoor, tot ritjes in auto's met rare fratsen, een weekje naar België voor testafnames, feestjes en opbeurende woorden in de groepsapp in reactie op acceptaties of afwijzingen van journals, tot sushi eten op kantoor om toch nog 'samen' aan een online conferentie deel te nemen. Inmiddels zijn wij niet alleen experts in ons vakgebied, maar kunnen we ook bolderkar-rijden en koffers of auto's inpakken als de beste! Samen hebben we op het moment dat ik dit dankwoord schrijf al 10 mooie artikelen gepubliceerd op de TRIXY data en ik weet zeker dat er nog snel meer zullen volgen. Lieve Nikki, jij mag natuurlijk ook niet ontbreken, heel leuk om samen op ontdekking te gaan wat het promoveren inhoudt en dat je nu mijn paranimf zal zijn!

Also, a big thanks to our colleagues in Denver! Thank you all for this amazing collaboration, your help with recruiting children and all the assessments. A special thanks to Nicole for her help on all the papers and for all the opportunities during my visit, it truly was an amazing experience!

Ook veel dank aan alle ouders en kinderen uit heel Nederland, België en Amerika die hebben deelgenomen aan de Pilot of TRIXY studie. Alle Bachelor- en Masterstudenten die met koffers vol materialen stad en land hebben afgereisd met auto's of het openbaar vervoer (zelfs naar dorpjes waar bussen maar twee keer per dag langskwamen!) om bij de deelnemers te komen, en naast dataverzameling ook een grote bijdrage hebben geleverd aan de verwerking van alle data, onwijs bedankt! In het bijzonder woord van dank aan Amber Dool, die na het afronden van haar masterproject en masterstage bij TRIXY bleef hangen om ons te ondersteunen om de

dataverzameling in te halen. Ook bedank ik mijn medepromovendi en andere collega's van NPOS voor de gezelligheid op de afdeling, het meedenken tijdens het AOI overleggen en de fijne lunches.

Tot slot, lieve familie en vrienden, dank voor jullie aanmoedigingen en interesse in mijn onderzoek en bevindingen (ook al was het voor sommigen van jullie nooit helemaal duidelijk wat een promovenda nou precies doet, 'ben je dan nog stéeds aan het studeren'?). Marjolein en Wouter (en Raymond en Alice) als oudere zus en broer heb ik veel aan jullie gehad en jullie zorgden ervoor dat ik van kleins af aan uitdagingen niet uit de weg ging (als jullie het konden, dan moest ik dat ook kunnen!). Milou, lief nichtje, als je later groot bent weet je dat misschien niet meer, maar jij hebt je 'tante Lien' veel geholpen door mij de ontwikkeling van een jong kind in het echt te laten zien in plaats van op papier te lezen. Daarnaast diende je ook soms als mijn eerste 'proefpersoon' als ik een nieuwe test moest afnemen, dankjewel! Pap en mam, jullie in het bijzonder bedankt voor jullie onvoorwaardelijke steun en hulp; zonder jullie had ik dit pad nooit bewandeld. Dank voor alles wat jullie mij hebben meegegeven, voor jullie vertrouwen en voor het stimuleren van mijn nieuwsgierigheid; er blijft immers altijd nog meer te ontdekken!