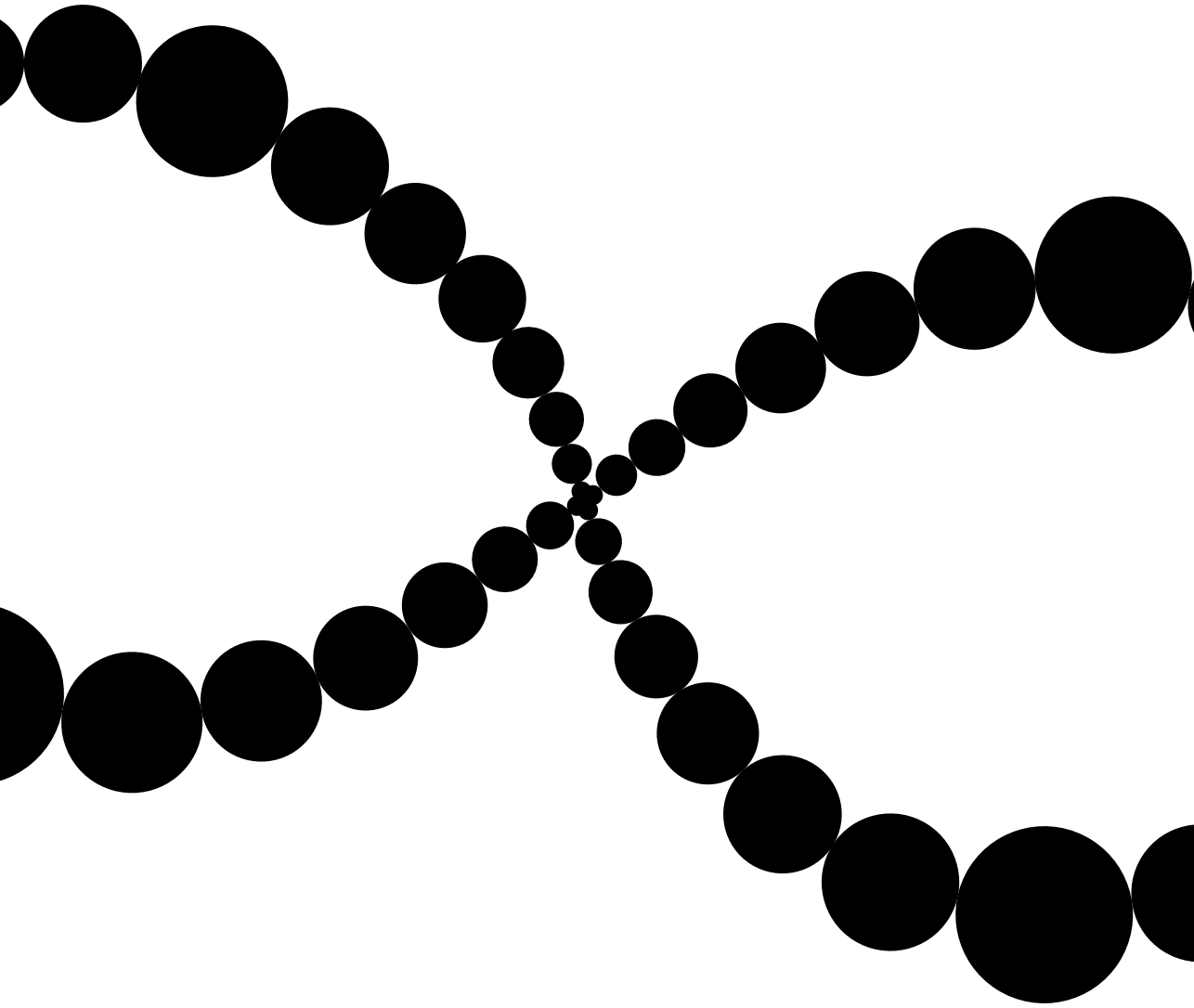
WHAT'S IN A FACE?

The early developmental impact of
Sex Chromosome Trisomies (XXX, XXY, XYY)
on social cognition



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CHAPTER 1

General introduction

Children grow up in a largely social environment. The ability to perceive, understand and react to social information is a prerequisite for participating in the social world, and is important for developing satisfying and lasting relationships, for being successful in school and workplace settings and consequently, to experience quality of life throughout the lifespan. The way children learn to navigate in the constantly changing dynamic social world is determined by complex interactions between neural, cognitive and behavioral activity. On physiological and neural level, there is a predisposition for the maturation of networks and functions in the brain that underpin the possibility to perceive and process relevant information. The network of interconnecting brain locations often referred to as the 'social brain', is comprising different structures that contribute to understanding and responding to social relevant information (Grossmann & Johnson, 2007). The maturation of the social brain is driven by genetic factors and stimulated by social interactions already from the first minutes of life. For example, newborns show an automatic orientation to faces and highly prefer to attend to face-like patterns (Reynolds & Roth, 2018). Due to brain maturation and interactions with others, children gradually develop the capacity to deal with more complex social information (Soto-Icaza, Aboitiz, & Billeke, 2015). Disruptions during development can contribute to difficulties in adaptation to demands of the social environment. These difficulties can in turn result in emotional and social problems like social isolation, reduced self-esteem and psychological distress. Vulnerabilities in social adaptive functioning may impact greatly on quality of life (e.g. Tobin, Drager, & Richardson, 2014). Also, difficulties in social adaptive functioning and impaired social (cognitive) development is associated to various forms of psychopathology (Besag & Vasey, 2019).

Understanding early pathways that shape the development of adaptive social functioning later in life is therefore important, not only in typical development, but especially in children that show developmental vulnerability with regard to social adaptive functioning. Sex Chromosome Trisomies (SCTs) are specific genetic conditions that may serve as naturalistic 'at risk' models of compromised social adaptive development, because they are associated with increased risk for social cognitive and behavioral vulnerabilities and related psychopathology (Reiss et al., 2000). Because genetic conditions such as SCT can be diagnosed in the prenatal period or in early infancy, this allows for the prospective study of social developmental pathways towards social vulnerabilities in children, adolescents and adults, providing

insights in etiological trajectories leading to behavioral vulnerabilities later on in life.

Sex Chromosome Trisomies (XXX, XXY, XYY)

SCTs are characterized by an extra X or Y chromosome compared to the typical karyotype of 46,XX in girls and 46,XY in boys, caused by a spontaneous error during early cell division (Leggett et al., 2010). Prevalence estimates of SCT vary from 1:650 to 1:1000 (Boyd et al., 2011). There is wide phenotypic variability among individuals with SCT, with a mild physical phenotype shared across SCT variants, with minimal atypical facial characteristics, tall stature, and low muscle tone. SCT is also associated with an increased risk of somatic, neurodevelopmental, educational, behavioral, and psychological difficulties during development and in adult life (Tartaglia et al., 2015). Neurodevelopmental challenges in childhood and adolescence include impairments in language development, social adaptive functioning, and executive functioning. Global intellectual functioning within SCT is variable, ranging from impaired to above average; mean intellectual functioning is in the average to low-average range (Urbanus, Van Rijn, & Swaab, 2020).

Social adaptation is among the key domains of vulnerability in SCT (Van Rijn, Urbanus, & Swaab, 2019; Urbanus, Van Rijn, & Swaab 2020; Tartaglia et al., 2020). On social behavioral level, SCT is associated with increased risk for vulnerabilities in social adaptive functioning including shyness, social immaturity, difficulties in forming interpersonal relationships, increased levels of social anxiety, social impulsivity, and impairments in underlying social cognitive mechanisms (see for reviews: Freilinger et al., 2018; Tartaglia et al, 2010; Ross et al., 2012; Urbanus, Van Rijn, & Swaab, 2020). SCT is associated with increased risk for symptoms of social impairments and Autism Spectrum Disorders (ASD; Van Rijn, 2019).

Relevance of studying genetic conditions

Genetic conditions such as SCT, with specific neurocognitive and neurobehavioral outcomes may help to understand mechanisms of developmental risk underlying complex behavioral phenotypes and psychopathology. In that way, SCT serves as a nunique naturalistic 'at risk' model of social neurodevelopment, and provides insights into developmental trajectories that may be more difficult to study in behaviorally

defined populations (Reiss et al., 2000). Interestingly, since SCT can be identified as early as prenatally in contrast to behaviorally defined classifications such as ASD, early developmental pathways can be studied prospectively from the first year of life. In order to understand early markers of 'at risk' social development and related social behavioral vulnerabilities later in life, it is relevant to study the impact of the relatively homogeneous genetic conditions of the SCTs on the social phenotype very early in life. Since social difficulties have a major impact on a broad range of developmental trajectories, including language acquisition, school readiness, peer acceptance and risk and resilience for developmental psychopathology, early identification of compromised social development may help to understand developmental pathways to social impairment and psychopathology. These insights may not only be beneficial for the SCT population but also for populations that only can be diagnosed later in life, based on behavioral dysfunction and symptoms, such as children with neurodevelopmental disorders like ASD.

Mechanisms underlying social impairment: brain behavior pathways

In order to identify early predictors for social impairment and to gain insight into early processes that indicate social risk pathways, it is helpful to investigate mechanisms that underlie social behavioral dysfunction. The study of brain behavior pathways in genetic conditions such as SCT may uncover new insights in mechanisms of developmental risk leading to social impairment. There is indeed evidence suggesting that an extra X or Y chromosome convergently impacts the maturation of the social brain (Raznahan et al., 2016; Hong & Reiss, 2014). Atypical brain maturation caused by SCT may be expressed in impaired development of social cognitive mechanisms, necessary to shape social and communicative behavior in everyday life (see Figure 1 for a graphical representation of the brain behavior model; Swaab, 2014). In this bottom-up brain behavior model, is has been hypothesized that behavior is made possible by the functioning of neural networks in the brain, shaped by interaction with the environment. Brain functions result in neurocognitive abilities, which in turn underlie behavior. The complex interplay of different cognitive functions facilitates adaptive behavior to environmental information. In the case of disruptions during development of certain behavior as for example associated to vulnerabilities in the social domain in SCT, it is assumed that one or more cognitive functions may not have developed optimally, resulting from adversities in brain maturation or

disruptions in brain function. From this bottom-up neuropsychological perspective, having an extra X or Y chromosome may compromise neurocognitive development and contribute to vulnerabilities in the interaction with the social environment, expressed in hampered social behavior, which may be associated to clinical diagnoses of neurodevelopmental disorders, such as ASD.

The understanding of the possible impact of SCT on neurocognitive developmental mechanisms driving the risk for social impairments provides opportunities for improving timely diagnostic screening, subsequent close monitoring of vulnerable children, and targets for early (preventive) support and intervention.

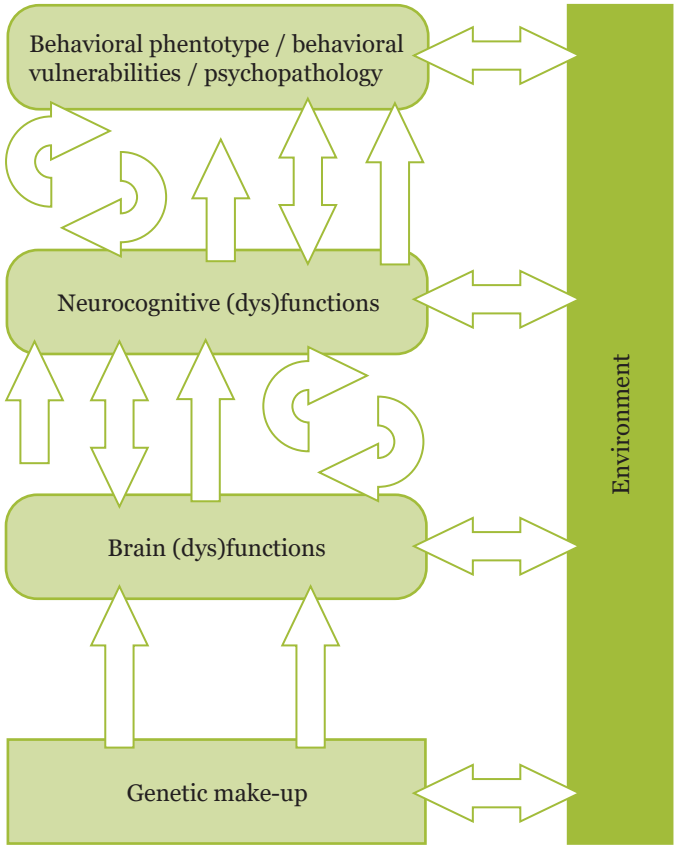

Figure 1. Brain behavior model (Swaab, 2014).

Social adaptation: concepts and definitions

As social adaptive functioning is among the key domains of vulnerability in SCT (Van Rijn, 2019; Urbanus, Van Rijn, & Swaab, 2020; Tartaglia et al., 2020), it is crucial to study early trajectories leading to risk for social compromised development in order to identify early markers for risk and targets for monitoring and intervention. Social adaptive functions refer to a spectrum of abilities that allow individuals to interact and communicate with each other, functions that are crucial for the forming of reciprocal and lasting relationships with others and for participating in society (as described in the SOCIAL model: Beauchamp & Anderson, 2010). In typical development, these social behavioral and cognitive abilities develop gradually in a temporal sequence of social milestones that may be needed to shape appropriate social functioning. In the first years of life, social cognitive development is characterized by the interactive emergence of several elements such as attention to social stimuli, eye contact, joint attention, recognition of faces and facial emotions, social perspective taking and the forming of a Theory of Mind. For an overview and definitions of common social concepts, such as social abilities and social cognition, as used in the present thesis, see Table 1.

Table 1. Definitions of social concepts as used in de present thesis.

Social concept	Definition
Social skills/ abilities	A wide range of abilities that develop to allow social cognitive processing. Adequate social skills or abilities allow us to interact and communicate with others, by predicting and understanding other people's intentions, feelings, emotions, and behaviors (Soto-Icaza, Aboitiz & Billeke, 2015)
Social cognition	Cognitive processes that allow us to interact with others and to understand other people's intentions, feelings, emotions and behaviors (Beauchamp & Anderson, 2010)
Social brain	Brain network whose function is associated with social functioning (Grossman & Johnson, 2007)
Social attention	The automatic and spontaneous visual orientation towards social relevant stimuli (Mundy& Neal, 2001)
Joint attention	The capacity to coordinate attention between interactive social partners with respect to objects or events in order to share an awareness of these objects or events (Nation & Penny, 2008)
Facial emotion recognition	The interpretation and encoding of emotional expressions from faces (Beauchamp & Anderson, 2010)
Theory of Mind	The ability to make the implicit assumption that the behavior of others is determined by their desires, attitudes and beliefs (Frith & Frith, 2003)

SCT and social adaptive functioning

The focus of research on psychological and cognitive characteristics of individuals with SCT has been broadened since the original descriptions of 47, XXY (Klinefelter Syndrome) by Dr. Harry F. Klinefelter in 1942 (Klinefelter, Reifenstein, & Albright, 1942) and 47,XXX and 47,XYY by Dr. Patricia A. Jacobs in 1959 and 1965 respectively (Jacobs et al., 1959; Jacobs et al., 1965). Initial newborn screening trials in the 1970's to 1990's focused on physical/somatic features, growth development and behavioral outcomes. Cross-sectional research in populations that were recruited through advocacy groups or in clinical settings in the decades thereafter reported impairments in the psychosocial domain, learning problems and risk for psychopathology (see for a detailed overview: Tartaglia et al., 2020). Although the phenotype of SCT is variable with some not having marked symptoms while others are more affected, these studies overall reported that individuals with SCT have social vulnerabilities including shyness, social immaturity, difficulties in forming adequate interpersonal relationships, and increased levels of social anxiety as well as social impulsivity (Bender et al., 1999; Otter, Schrander-Stumpel, & Curfs, 2010; Ross et al., 2012; Van Rijn et al., 2014a).

The severity of social impairment in individuals with SCT is illustrated by reports of increased risk for social problems that are typical in ASD symptomatology. Compared to a worldwide prevalence rate of ASD of 0.6% in the general population (Elsabbagh et al, 2012), prevalence of ASD has been shown to be significantly higher in SCT. On average across studies, depending on the ascertainment methods, diagnostic measurements and criteria used, full criteria of ASD are met in about 15% (range 10.8-20%) of individuals with 47,XXX, 18% (range 10-27%) of individuals with 47,XXY and 30% (range 19-43%) of individuals with 47,XYY (see for a review: Van Rijn, 2019). Noteworthy is that these studies reported more symptoms on the social communication and interaction domain of ASD, as compared to the domain of restricted, stereotyped and repetitive behaviors (see for example for boys and girls with an extra X chromosome: Van Rijn et al., 2014a). These findings indicate a specific profile of social impairments that are typical for ASD in individuals with SCT. From a bottom-up perspective, having an extra X or Y chromosome may compromise social development and contribute to symptoms and clinical diagnoses of neurodevelopmental disorders such as ASD. Identifying early profiles and severity of ASD symptoms in genetic conditions such as SCT, may therefore give insights

of developmental pathways leading to complex behavioral phenotypes. For the diagnostic criteria of ASD according to the Diagnostic and Statistical Manual of Mental Disorders - 5th version (DSM-5; American Psychiatric Association, 2013), see the supplementary materials (Box 1).

Although the vulnerability for communication and social interaction problems that is typical for ASD is clearly higher in SCT than in the general population, so far studies that focused on the understanding of underlying neurocognitive mechanisms related to these behavioral outcomes are scarce. Only vulnerabilities in the language domain were comprehensively studied in SCT, and developmental language problems are hypothesized to be a cognitive mechanism contributing to social impairment in SCT (Haka-lkse, Stewart, & Cripps, 1978; Ross et al., 2008; Ross et al., 2009; Bishop et al., 2011). Two recent reviews suggest that it is important to further investigate impairments in a broader range of essential social cognitive mechanisms in SCT, as they may be key aspects underlying risk for social behavioral vulnerabilities and related psychopathology in general (Urbanus, Van Rijn, & Swaab, 2020; Van Rijn, 2019).

Previous research that studied social cognitive functioning in SCT is limited to studies with broad age-range samples from school-age into adolescence and adulthood. With respect to social cognitive function, so far we learned that individuals with SCT have deficits in attending to social cues. To illustrate, research in adult men with 47,XXY, and in boys and girls with an extra X chromosome (47,XXX and 47,XXY) showed reduced attention to eyes and no typical tendency to first fixate on the eyes, both during the processing of static facial expressions (Van Rijn, 2015), and during orientation on dynamic presentation of faces emotion (Van Rijn et al., 2014b). Research also revealed that boys and adults with 47,XXY have increased risk for difficulties with facial affect recognition (Samango-Sprouse et al., 2018; Van Rijn, de Sonneville, & Swaab, 2018). School-aged children and adolescents with an extra X chromosome (47,XXX; 47,XXY) also show impairments in identifying angry facial expressions (Van Rijn et al., 2014b). Also, research showed that Theory of Mind in school-aged boys and girls with 47,XXY and 47,XXX is less well developed compared to typical developing controls (Van Rijn et al., 2014b; Melogno et al., 2019). Although further research is needed to learn more about mechanisms of social cognition in SCT, it is evident that there is a risk of disruption in social cognitive function in SCT.

Clinical relevance of studying early social developmental pathways in SCT

Psychosocial research focusing on age ranges from childhood into adulthood has reported adverse effects of SCT on quality of life, because of medical, psychological and social implications of the genetic condition (Close et al., 2015; Turriff, Levy, & Biesecker, 2011; Turriff et al, 2017; Wallentin & Gravholt, 2015). Besides this general impact of SCT on quality of life, individuals with SCT described challenges in finding health care professionals who have in depth knowledge about SCT. They often reported extensive search before receiving a diagnosis and difficulties with seeking support for coping with the vulnerabilities they face, which further impacts quality of life.

Due to advances in noninvasive prenatal assessment technology, the number of prenatal diagnoses of SCT is rapidly increasing (Tartaglia et al., 2020). Given this, there is the opportunity to prospectively investigate early developmental pathways towards specific vulnerabilities in children, adolescents and adults with SCT. However, there is not only the opportunity to prospectively investigate early development, but also the pressing need to gather knowledge on the development of children with SCT, which can be used for genetic counseling and psychoeducation. Considering the known vulnerabilities in social adaptation and the impact of these on quality of life, the research field of SCT is in need of prospective studies in order to identify early markers that may indicate 'high risk' in social development, as well as insight in developmental mechanisms that should be targeted in early support and intervention, and should be addressed in psychoeducation for caretakers in order to optimize the environment of children at genetic risk. The more we learn about the earliest risk and protective factors predictive for behavioral outcome, the more we are able to design effective treatment programs. The identification of targets for early monitoring and intervention can support the development of evidence-based care and improve health and neurodevelopmental outcome for this growing population of children with SCT and other children with the same vulnerabilities.

Aims and outline of the present thesis

The aim of the present thesis is to explore the impact of SCT (XXX, XXY, XYY) on early social adaptive behavior and social cognitive developmental mechanisms, and

to identify early markers of high risk in social development. We aim to investigate social adaptive functioning and underlying social cognitive mechanisms in a relatively large and international group of young children with SCT, aged 1-8 years old, compared to typical developing peers. The studies described in the present thesis are part of the TRIXY Early Childhood Study (Leiden, The Netherlands) that prospectively investigates neurodevelopmental risk in young children with SCT. In these studies, we include more than 100 children with SCT and an age-matched typical developing group of children with the same size. Recruitment and assessment took place at two sites: the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, The Netherlands, and the eXtraordinary Kids Clinic at the Children's Hospital in Colorado, Denver, USA. The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping. Our study samples consist of a mix of prenatal and postnatal diagnosed children, and three different recruitment cohorts were identified: (1) prospective follow-up after prenatal diagnosis, (2) information seeking parents after prenatal or postnatal diagnosis, and (3) clinically referred cases after prenatal or postnatal diagnosis.

Based on the knowledge of convergent effects of the X and Y chromosomes on brain areas key to social cognition (Raznahan et al., 2016), and on earlier studies which showed no XXX/XXY differences in social behavior and social cognition (see for example: Van Rijn et al., 2014a; Van Rijn et al., 2014b), we studied the three separate karyotypes (XXX, XXY, XYY) within SCT as one study sample.

In Chapter 2 and Chapter 3 the question is addressed how SCT impacts the development of early social adaptive behavior. As the first years of life is a period in which the social brain network rapidly matures and specializes, this period serves as a key period to acquire social emotional developmental milestones (Grossmann & Johnson, 2007). In order to identify early markers of 'at risk' social development and related social behavioral vulnerabilities later in life, it is important to study the impact of SCT on the social behavioral phenotype very early in life. We focused on the impact of SCT on early social communication abilities such as the initiating of reciprocal communication, studied during structured behavior observations, and we investigate daily life social emotional milestones (Chapter 2). In daily life, social communication abilities are important to support sensitive social interaction: they

shape the capacity of the child to engage with others, to comprehend emotional expressions of others, and to elaborate upon a range of feelings in social interactions (Bayley, 2006). Therefore, in the next study, we were especially interested whether and how SCT impact the way young children shape social behavior during observed social interactions (Chapter 3).

The severity of the impact of SCT on social behavioral development is illustrated by increased risk for social impairments that are typical for ASD symptomatology. Previous studies reporting on ASD symptomatology in SCT focused on populations with broad age-ranges, including participants from middle childhood to adulthood (see for a review: Van Rijn, 2019). We aimed to study the possible impact of SCT on the severity and profiles of ASD symptoms during the first eight years of life (Chapter 2).

In Chapter 4 and 5 the question was addressed whether an impact of SCT also could be found on cognitive mechanisms of social behavior, i.e. early social cognitive mechanisms underlying social behavior. Knowledge about the early impact of SCT on social cognitive mechanisms is important to understand how behavioral vulnerabilities may arise from information processing difficulties, to disentangle different etiological pathways leading to social impairments and psychopathology, and to identify early markers for monitoring and tailored (preventive) support and intervention programs. We explored basic and essential social cognitive mechanisms like attention to faces and eyes, social orienting and joint attention, by using eyetracking. We also investigated more complex social cognitive abilities such as facial emotion recognition and Theory of Mind. As there have been, as far as we know, no studies investigating social cognition in children with SCT younger than eight years old, these studies aim to understand early developmental pathways leading to social vulnerabilities (see for reviews: Van Rijn, 2019; Urbanus, Van Rijn, & Swaab, 2020). Cross-sectional age differences were explored to further understand the impact of SCT on developmental pathways in social cognition. We also aimed to investigate early pathways to social impairment as expressed in ASD symptoms, by exploring the predictive value of early joint attention for social behavior at one year follow-up. Based on the expected relevance of the X and Y chromosome for the development of neural networks supportive of social functioning (Hong & Reiss,

2014; Raznahan et al., 2016), we hypothesized that 1-8 year old children with SCT might show less well developed social behavioral and cognitive abilities, as compared to their age matched peers. By identifying the effect of specific karyotype-subtype and possible recruitment bias, the studies presented in the current thesis allow for an investigation of phenotypic differences within the SCT group.

The final study (Chapter 7) focused on the efficacy of a neurocognitive training program in 4-8 year old children with SCT. This neurocognitive training program was targeted at improving a core aspect of social cognition, the understanding of social cues from facial expressions. We studied outcomes of this training program on emotion recognition, Theory of Mind and social orienting in children with SCT, compared to a waiting list SCT group, and a typically developing control group. Feasibility of the training is evaluated by parents and children.

In Chapter 8, we summarize and discuss the main findings of the present thesis, including reflections about future research avenues and clinical implications.

Supplementary materials

Box 1. DSM-5 criteria for Autism Spectrum Disorder.

Autism Spectrum Disorder (ASD)

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history (examples are illustrative, not exhaustive, see text):

1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions.
2. Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication.
3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

B. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history (examples are illustrative, not exhaustive; see text):

1. Stereotyped or repetitive motor movements, use of objects, or speech (e.g., simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases).
2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonverbal behavior (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat food every day).
3. Highly restricted, fixated interests that are abnormal in intensity or focus (e.g, strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interest).
4. Hyper- or hypo reactivity to sensory input or unusual interests in sensory aspects of the environment (e.g.,

apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement).

C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities or may be masked by learned strategies in later life).

D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

E. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level.

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CHAPTER 2

Early impact of X and Y chromosome variations (XXX, XXY, XYY) on social communication and social emotional development in 1-2 years old children

Bouw, N., Swaab, H., Tartaglia, N., Jansen, A. C., & Van Rijn, S. (2022). Early impact of X and Ychromosome variations (XXX, XXY, XYY) on social communication and social emotional development in 1–2-year-old children. *American Journal of Medical Genetics Part A*.

Abstract

Objective: Sex Chromosome Trisomies (SCT) are characterized by an extra X or Y chromosome (XXX, XXY, XYY). This study aims to investigate early signs of social communication and social emotional development in very young children with SCT. 34 children with SCT (aged 12-24 months) were included in this study, as well as 31 age-matched controls.

Methods: Social communication was measured with structured behavior observations according to the Early Social Communication Scales, and social emotional developmental level with the Bayley Social Emotional parental questionnaire. Recruitment and assessment took place in the Netherlands and in the USA.

Results: On average, 12-24 month old children with SCT showed difficulties with early social communication, more so in responding to others as compared to initiating social communications. During social interactions, children with SCT made less frequent eye contact, compared to controls. Also, difficulties in acquiring social emotional milestones were found in 1-year old children with SCT, with 44% of the children having social emotional vulnerabilities in the borderline or extremely low range, compared to typically developing children. In this cohort, no significant predictive effects of karyotype-subtype (XXX, XXY, XYY) were found.

Conclusions: Already from a very early age, SCT can be associated with increased risk for vulnerabilities in adaptive social functioning. These findings suggest that SCT impact the maturation of the social brain already from an early age, and stresses the importance of early monitoring and (preventive) support early social development in young children with SCT.

Introduction

Sex Chromosome Trisomies (SCT) are specific genetic conditions that may serve as naturalistic 'at risk' models of neurodevelopment, because they are associated with increased risk for neurobehavioral difficulties and psychopathology (Reiss, Eliez, Schmitt, Patwardhan & Haberecht, 2000). Because such genetic conditions can be diagnosed in the prenatal period or in infancy, this allows for the prospective study of developmental pathways towards specific vulnerabilities in children, adolescents and adults with SCT. SCT are characterized by an extra X or Y chromosome compared to the typical karyotype of 46,XX in girls and 46,XY in boys. Prevalence estimates of SCT vary from 1:650 to 1:1000 (Boyd, Loane, Garne, Khoshnood & Dolk, 2011). Knowledge about the impact of SCT on the neurocognitive and neurobehavioral phenotype is growing, and it is known that a disproportional high percentage of genes on the X chromosome play a role in human cognition and brain development (Zechner, Wilda, Kehrer-Sawatzki, Vogel, Fundele & Hameister, 2001). There is evidence suggesting that an extra X or Y chromosome convergently impact the maturation of a distributed and interactive network of cortical and subcortical components underlying adaptive social cognitive functioning, often described as the 'social brain' (Raznahan et al., 2016; Hong & Reiss, 2014). Anatomical brain structures related to social functioning that seem to be affected by SCT include the posterior insula, the anterior cingulate, the medial prefrontal cortex, the superior temporal sulcus, and the orbitofrontal cortex (Raznahan et al., 2016).

Social brain alterations in SCT fit with the increased risk for deficiencies in the processing of social information and with difficulties in social emotional development, like problems in understanding social emotional information, and in showing social adaptive behavior during interactions with others, that have been observed in school-aged children, adolescents and adults with SCT. Although the phenotype of SCT is variable with some not having marked symptoms while others are more affected, studies that evaluated several domains of social adaptive functioning in individuals with SCT found social vulnerabilities including shyness, social immaturity, difficulties in forming adequate interpersonal relationships, increased levels of social anxiety and social impulsivity, and impairments in underlying social cognitive mechanisms (see for reviews: Freilinger, Kliegel, Hänig, Oehl-Jaschkowitz, Henn & Meyer, 2018; Tartaglia, Cordeiro, Howell, Wilson & Janusz, 2010; Ross et al., 2012; Urbanus, Van Rijn & Swaab, 2020). The severity of social behavioral and social

cognitive impairments in school-aged children, adolescents and adults with SCT is illustrated by an increased level of clinical diagnoses of Autism Spectrum Disorder (ASD). Average percentages of ASD classifications across studies vary from 15% (range 10.8-20%) in individuals with 47,XXX; 18% (range 10-27%) in individuals with 47,XXY; to 30% (range 19-43%) in individuals with 47,XYY (Van Rijn, 2019).

In order to identify early markers of 'at risk' social development and related social behavioral vulnerabilities later in life, it is important to study the impact of SCT on the social behavioral phenotype very early in life. The first study on social behavioral characteristics in children with SCT aged 1-5 years found social behavioral problems already at this age (Urbanus, Swaab, Tartaglia, Cordeiro & Van Rijn, 2020). Since social difficulties have a major impact on a broad range of areas of development, including language acquisition, school readiness, peer acceptance and risk and resilience for developmental psychopathology, early identification of compromised social development in young children with SCT can help understand developmental outcome and finding targets for preventive intervention (Rao, Beidel & Murray, 2008).

Early social development is marked by the growing ability to co-ordinate eye contact and to engage in reciprocal social emotional interactions (Soto-Icaza, Aboitiz & Billeke, 2015). Already in the first hours of life, infants prefer to look at faces that engage in eye contact (Farroni, Csibra, Simion & Johnson, 2002). Making eye contact with others is a basic biological mechanism, essential for social communication, as the visual information in eyes are important sources of information used to understand communicative goals and emotional states of others (Senju & Johnson, 2009). Thereafter, young children develop specific communication skills underlying socially adaptive behavior, in which the following elements are of particular importance: following eye gaze and conventional gestures of others in order to achieve shared attention to an object or event (i.e. joint attention), the ability to communicate own beliefs and desires and to react to the desires of others, and being part of reciprocal social interactions. These specific communication skills involve the use of eye contact, and serve to hold and coordinate attention between interactive social partners (Mundy, 2003/2013). In naturalistic daily life settings, social communication skills are linked to significant accomplishments of broad social emotional functioning: they shape the capacity of the child to engage with others, to comprehend emotional

expressions of others, and to elaborate upon a range of feelings in social interactions (Bayley, 2006).

As the first years of life is a period in which the social brain network rapidly matures and specializes, this period serves as a key period to acquire social emotional developmental milestones (Grossmann & Johnson, 2007). Therefore, it is especially important to focus on social behavioral abilities during that stage of early childhood as a means of identifying early markers of an 'at risk' social development. The current study aims to provide in that by investigating the impact of SCT on the early communicative and social emotional phenotype in a group of children, aged 12-24 months.

This study may have specific benefit to the clinical care of individuals with SCT. As SCT is being increasingly identified during pregnancy with recent technical advances of non-invasive prenatal screening (i.e. the noninvasive prenatal screening test; NIPT), the population of infants with prenatal diagnosis of SCT is rapidly growing (Tartaglia et al., 2020). The study of very young children with SCT supports the identification of children with SCT at risk for neurobehavioral difficulties and psychopathology later on in life, and will give insight in potential targets for monitoring, early prevention and intervention.

Taken together, this study aims to investigate the impact of SCT on structured observations of early social communication and parental report of social emotional development in 1-year old children. In addition to these main research questions, predictive effects of karyotype-subtype (XXX vs. XXY vs. XYY) on early communicative skills and social emotional skills were studied. Based on the relevance of the X- and Y-chromosome for the development of neural networks supportive of adaptive social functioning, we hypothesized that 1-year old children with SCT might show less well developed social emotional and communicative skills, compared to a control sample.

Methods

Participants

The present study is part of a larger ongoing project (the TRIXY Early Childhood Study - Leiden the Netherlands), which includes children with SCT and nonclinical

controls. A group of 34 children with SCT aged 12-24 months, was included in this study (M_{age} = 1.39, SD = 0.36), as well as 31 age-matched controls (13 boys; M_{age} = 1.53, SD = 0.28). Mean age did not significantly differ between groups (t (63) = 1.69, p = .096). The SCT group consisted of 6 girls with 47,XXX (17.6%), 20 boys with 47,XXY (58.8%) and 8 boys with 47,XYY (23.5%). Gender distribution differed between the SCT and control group (χ^2 (1) = 11.37, p = .001). Recruitment and assessment took place on two research sites: the Trisomy of the X and Y chromosomes (TRIXY) Expert Center in 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 and pediatrics departments (from the Netherlands, Dutch speaking parts of Belgium and the USA), as well as through patient-advocacy groups and social media postings. One girl with XXX, 5 boys with XXY and 3 boys with XYY were recruited and assessed in the Netherlands. Five girls with XXX, 15 boys with XXY and 5 boys with XYY were recruited and assessed in the USA. Karyotype distribution did not differ between the two research sites (χ^2 (2) = .82, p = .664). Thirty-two children (94.1%) were diagnosed prenatally, and two children postnatally (5.9%; 1 girl with XXX, 1 boy with XXY). 11 out of 20 boys with 47,XXY had received testosterone treatment (55%).

The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping. For the SCT group, recruitment strategy was assessed, and three subgroups were identified: (1) 'active prospective follow-up', which included families who were actively followed after prenatal diagnosis (67.6% 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 (26.5% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development (5.9% of the SCT group). The control group was recruited from the western part of the Netherlands, and approached with information brochures about the study. All participants were Dutch or English speaking, had normal or corrected-to-normal vision, and did not have a history of traumatic brain injury or hearing loss. 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 ~1 in 1000, the risk of having one or more children with SCT in the control group was considered

minimal and acceptable.

Parental education and age of the primary caregiver were assessed. Parental education was assessed according to the criteria of Hollingshead (Hollingshead, 1975). Scores of this scale include: 0 (no formal education), 1 (less than seventh grade), 2 (junior high school), 3 (partial high school), 4 (high school graduate), 5 (partial college or specialized training), 6 (standard college/university graduation), and 7 (graduate/ professional training). 98% of all parents indicated that their child has a second caregiver. If two parents were available, level of education was averaged over both parents. A Pearson χ^2 test was performed to investigate possible differences in parental education distribution between the SCT and control group. No difference was found (χ^2 (8) = 12.04, p = .149), indicating equal parental education in the SCT group (M = 5.96, SD = 0.88), and the control group (M = 5.47, SD = 1.32).

Measurements

Background measures: level of cognitive and language development. In order to measure general cognitive functioning and language development the Bayley-III (composite score of the cognitive and language subscale (Bayley, 2006) was administered in the original English version and the translated Dutch version (Bayley-III-NL; Steenis, Verhoeven, Van Baar, 2012). This test aims to measure cognitive and language skills in children from 1 to 42 months of age.

Early social communication: ESCS. The Early Social Communication Scales (ESCS; (Mundy, 2003/2013) is a systematic behavior observation of a structured 20-min play situation, designed to measure key early social communication skills that are usually acquired in the first 30 months of life. Following the procedures described by Mundy, (2003/2013) the behavior ratings of specific defined responses during fixed time intervals were scored by trained independent raters. Raters were not involved in the assessment, and blind to the child's group membership and karyotype. Three distinct and mutually exclusive social communicative subscales were scored based on the videotaped session: joint attention (JA), behavioral requests (BR) and social interaction (SI). A distinction was made between initiating social communication and responding to social communication, resulting in six domains of early social communication. Variables of interest were the total scores on the six communicative

Table 1. Description of early social communication coded behavior during social interactions (based on Mundy, 2003/2013).

Joint Attention		
Initiating Joint Attention	Eye contact	Child makes eye contact with examiner while manipulating or touching an inactive mechanical toy.
	Alternate	Child alternates looking at active mechanical toy or a toy in their hand and the examiner's eyes.
	Point	Child extends index finger toward toy within reach or to part of the room (e.g. posters).
	Show	Child extends toy toward the examiner's face.
Responding to Joint Attention	Look	Child turns head and eyes in the direction of the examiner's pointing gesture, or to the appropriate area of a book.
Behavioral Requests		
Initiating Behavioral Requests	Eye contact	Child makes eye contact with examiner after a toy is removed from the child.
	Reach	Child extends arm toward an out of reach toy.
	Appeal	Child reaches to toys which are out of reach and makes eye contact with the examiner.
	Point	Child points to toys that are out of reach.
	Give	Child pushes toy toward examiner or holds an object out toward the examiner's hand or body.
Responding to Behavioral Requests		Child responds to gestural and verbal requests of the examiner.
Social Interactions		
Initiating Social Interactions	Initiates turn-taking	Child initiates game by rolling ball or car to the examiner.
	Tease	Child engages in a prohibited activity (e.g. throwing toys from table) while smiling at the examiner.
Responding to Social Interactions	Eye contact	Child makes eye contact with examiner after examiner has tickled the child.
	Act	Child vocalizes or bangs the table or child reaches to examiner after the examiner has tickled the child.
	Appeal	Child reaches to examiner and makes eye contact after being tickled.
	Turn taking	Child takes turns with examiner throwing the ball or rolling the car.

subscales in addition to the frequency of eye contacts of the child during three of the ESCS domains (Initiating Joint Attention, Initiating Behavioral Requests, and Responding to Social Interactions). See Table 1 for a description of the nonverbal communicative behaviors within the six subscales of early social communication.

Social-emotional development: Bayley SE. The Bayley Social Emotional Questionnaire (Bayley SE; Bayley, 2006) consists of 21 or 24 items (dependent on the age of the child), and measures the acquisition of functional social-emotional milestones in naturalistic settings that broadly represent social-emotional patterns and developmental accomplishments. The Bayley-SE questionnaire was administrated in the original English version (Bayley, 2006), and the translated Dutch version (Bayley SE-NL; Van Baar, Steenis, Verhoeven, & Hessen, 2014). The items of the Bayley SE assess the attainment of age-related milestones of the child, namely the ability to engage and use various emotions, expressions and experiences, as well as the comprehension of a range of social and emotional signals and to understand and react to a feelings of others with words, gestures or imitations. Examples of items are: 'Shows you that he or she understands your actions or gestures by making an appropriate gesture in return (e.g. make funny face back at you, looks at something you point to, stops doing something when you shake your head and use a firm voice to say "No!!" or smiles and does more of something when you nod with a big smile and say "Yes!)' and 'Uses many consecutive actions in a back-and forth way to show you what he or she wants or to have fun with you (e.g. smiles, reaches out for a hug, and, when you hug, takes your hat, puts it on his or her head, and smiles proudly OR takes your hand, leads you to the refrigerator, tugs on the handle, and, after you open it, points to something he or she likes, such as food, a bottle of juice, or milk.'

The primary caregiver rates his/her child on a 5-point Likert scale ranging from 1 'none of the time' to 5 'all of the time'. Raw scores were used to compare the children with SCT and the controls. Based on the guidelines of the Bayley 3rd Edition Manual, the total raw scores of the Bayley SE were converted to a composite score, ranging from 55 to 145 with a mean of 100 and standard deviation of 15 points in the norm population. The composite scores were labeled as being in the average range (composite score > 90), the 'Borderline/monitoring' range (composite score 70-89), and the 'Extremely low/at risk' range (composite score < 70; Weiss, Oakland & Aylward, 2010). The Bayley SE has high internal consistency and test-retest reliability

(Weiss, Oakland & Aylward, 2010), and the original validation study demonstrated that the Bayley SE distinguishes significantly between clinical groups including children with genetic syndromes or developmental disorders (Bayley, 2006).

Study procedures

Signed informed consent was obtained from the parents of all participating children, according to the declaration of Helsinki. 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. Assessment took place at different sites (Colorado USA, and the Netherlands) 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). Administration of the ESCS always took place after administration of the Bayley-III in order to prevent familiarity differences to interfere with the test scores. During the ESCS, the child was seated at a table across from a familiar examiner (see Figure 1 for the set-up of the assessment room). Verbal interactions were kept to a minimum during the ESCS. The 20-min structured assessment was videotaped, with full face view of the child and profile view of the experimenter. The parent questionnaire was completed by the primary caregiver of the child, either in Dutch or in English.

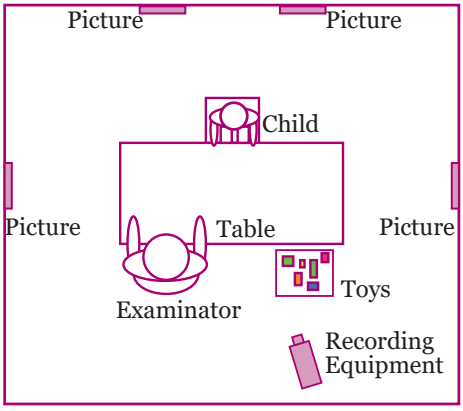

Figure 1. Set-up assessment room ESCS administration (adapted from: Mundy, 2003/2013).

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25. Independent *t*-tests and MANOVAs were carried out to test outcome differences between gender (boys vs. girls) and between research sites (USA vs. NL). Independent *t*-tests were used to test for differences between the SCT and control group for cognitive and language development. Multivariate analyses of variance (MANOVAs) were used to test differences on early social communication, with the scores on the six subscales of the ESCS (IJA, RJA, IBR, RBR, ISI, RSI) as dependent variables and research groups (SCT, control) as independent variable. Independent *t*-tests were used to test differences on social emotional functioning, with the scores on Bayley SE as dependent variable and research groups (SCT, control) as independent variable. Multivariate and univariate analyses of Covariance (ANCOVA and MANCOVA) were used to control for global cognitive and language level. To test SCT vs. control differences in eye contact, three separate independent t-tests were used with number of eye contacts on three subscales of the ESCS (IJA, IBR, RSI) as dependent variables. Pearson's correlation analyses were used to test the association between early social communication and daily life social emotional development in the SCT group. Linear regression with dummy coding were used to test for the effect of karyotype-subtest and recruitment strategy on the scores on Bayley SE and ESCS. Level of significance was set at $p < .05$, two-tailed. Effect sizes were calculated with Cohen's *d* or partial η^2 when applicable.

Results

Comparison between gender and research sites

No differences were found for social communication on the six subscales of the ESCS ($F(6, 26) = 1.34, p = .275$), and social emotional functioning measured with the Bayley SE ($t(29) = -1.95, p = .061$) between control boys and girls. Therefore, data between boys and girls were collapsed across gender groups. Also, no differences between the research sites (The Netherlands, US) were found for social communication ($F(6, 26) = 1.90, p = .120$), and social emotional development ($t(32) = -0.94, p = .355$). Based on this, all SCT data were collapsed across sites.

Background measures: cognitive and language development

The Bayley-III was successfully completed by 64 children. Global cognitive development did not differ between the SCT (M = 99.85, SD=13.26) and the control group (M = 99.71, SD=13.98; t (62) = 0.41, p = .968). However, language development did differ between the SCT (M = 95.94, SD=15.83) and the control group (M = 110.19, SD=13.32; t (62) = -3.89, p <.001).

Structured behavior observations of early social communication

Data quality. The ESCS was successfully completed by 63 children (two children were not able to complete the task). Although all children received at least 14 out of 18 trials of the active wind-up toys and hand-operated toys, some trials of four children are not administrated (e.g. because the child was crying) or are excluded due to technical aspects (e.g. experimenter obscured camera angle). Therefore, for these children the mean value of the coded social behaviors on other trials was used in place of these missing data (= mean substitution; Kang, 2013). Interrater reliability was measured based on a subsample of ten participants, and showed an intraclass correlation coefficient (ICC) of 0.84-0.96 (for the ESCS domains collapsed together) which is considered excellent reliability (Cicchetti & Sparrow, 1981).

Early social communication and age. Within the SCT group, social communication was positively correlated with age on domains of Initiating Behavioral Requests, Responding to Joint Attention, and Responding to Social Interaction, indicating better social communicative skills in older children. No correlation with age was found for the other three ESCS domains. See Table 2 for exact Pearson's r, and p-values.

Table 2. Early social communication in children with SCT (aged 12-24 months), compared to control group (Mean, *SD*).

	SCT	**Control**	*p*-value	Group differences	Effect size (η_p^2)	Correlation with age within the SCT group
Early social communicative domains	*n* = 33	*n* = 30				
Initiating Joint Attention	17.30 (9.68)	18.80 (9.35)	.536	SCT = control		*r* = .169, *p* = . 346
Initiating Behavioral Requests	13.88 (7.81)	18.67 (10.59)	.044	SCT = control	.07	*r* = .407, *p* = .019
Initiating Social Interactions	0.45 (0.62)	0.63 (0.67)	.274	SCT = control		*r* = .225, *p* = . 207
Responding to Joint Attention	.65 (.31)	.85 (.19)	.004	SCT = control	.13	*r* = .487, *p* = . 004
Responding to Behavioral Requests	16.27 (8.20)	22.47 (5.63)	.001	SCT = control	.17	*r* = .-.051, *p* = . 780
Responding to Social Interactions	11.61 (6.30)	14.53 (6.87)	.083	SCT = control	.05	*r* = .454, *p* = . 008

Note: SCT = Sex Chromosome Trisomies.

Early social communication: SCT vs. controls. Differences in early social communication between the SCT and control group were analyzed with a MANOVA, using Pillai's trace, with the six subscales of the ESCS as dependent variables. A significant difference between the SCT and control group was found (F (6, 56) = 3.28, p = .008; η_p^2 = .26). Post-hoc ANOVA tests on the outcome variables revealed a significant group effect on three of the six subscales of the ESCS: Initiating Behavioral Requests (p = .044; η_p^2= .07), Responding to Joint Attention (p = .004; η_p^2 = .13), and Responding to Behavioral Requests (p = .001; η_p^2 = .17). The multivariate significant difference between groups remained even whe n cognitive and language level is added as covariate (F (6,53) = 1.05, p = .043, η_p^2 =.21). These results indicate more difficulties in early social communicative behaviors in 1-year old children with SCT compared to the control group, with medium effect sizes. Descriptive statistics and post-hoc effects can be found in Table 2.

Eye contact: SCT vs. controls. Following the procedures described by Mundy (2003/2013), number of eye contact was coded under three of the six social communicative domains (Initiating Joint Attention, Initiating Behavioral Requests and Responding to Social Interactions). Differences in eye contact between the SCT and control group are analyzed using three independent t-tests. A significant difference was found between the SCT and control group for eye contact during Responding to Social Interactions (t (61) = -2.55, p = .013, Cohen's d = 0.64), indicating less frequent eye contact in the SCT group, compared to controls. No differences were found between the SCT and control group for eye contact during initiating of social communication: Initiating Joint Attention (t (61) = -3.44, p = .732) and Initiating Behavioral Requests (t (61) = -1.49, p = .140). See Figure 2 for exact means.

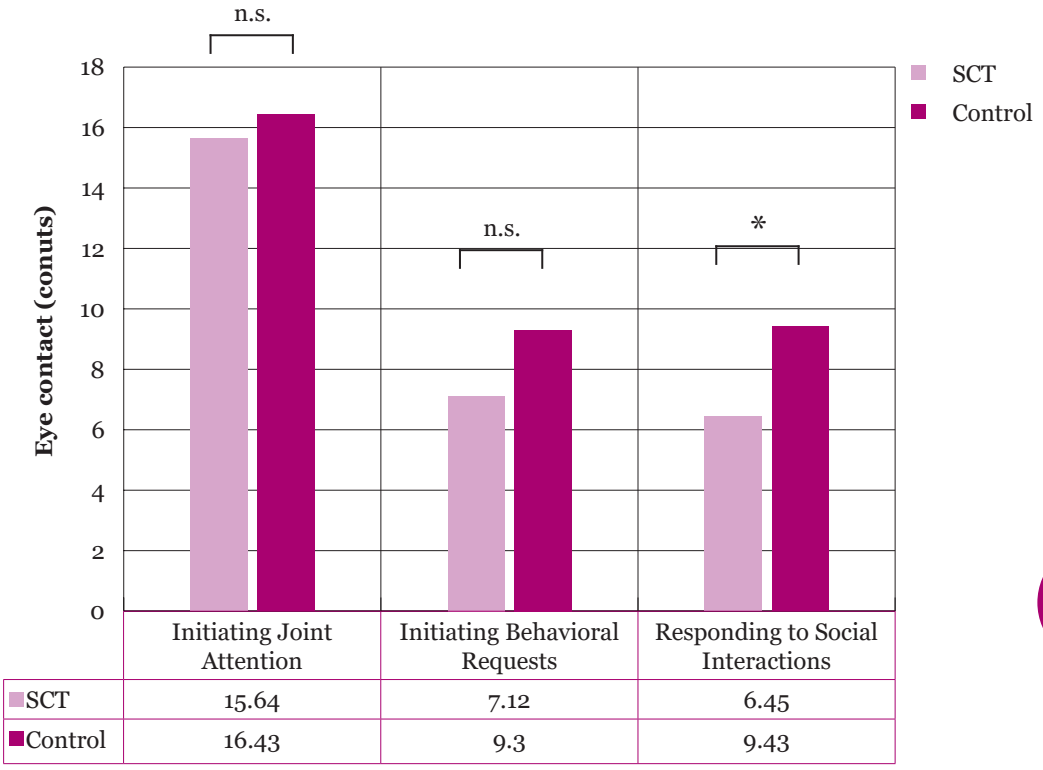

Figure 2. Eye contact for the SCT and control group.
Note: * = p <.05, n.s. = non-significant; SCT = Sex Chromosome Trisomies.

Social emotional development: SCT vs controls

Within the SCT group, social emotional functioning was positively correlated with age (r = .810, p <.001). An independent t-test with Bayley SE raw score as dependent variables revealed a difference in overall social emotional functioning between the SCT (M = 81.68, SD=16.05), and the control group (M = 93.32, SD=13.71; $t(63)$ = -3.13, p = .003, Cohen's d = 0.78). On average, parents reported that children with SCT have more social emotional difficulties compared to the control group, with a medium effect size. The significant difference between groups remained, even when cognitive and language level was added as covariate (F (1,60) = 12.41, p = .001, η_p^2 =.17). When evaluating scores normalized for age, for overall social emotional functioning, 56% of the SCT group scored in the average range, 38% scored in the borderline/monitoring range, and 6% in the extremely low/at risk range.

Association between early social communication and overall social emotional development

To explore the associations between early social communication behaviors and daily life social-emotional development within the SCT group, Pearson's correlations were calculated. Significant positive correlations were found between three early social communicative domains and social emotional development (initiating behavioral requests, r = .453; responding to joint attention, r = .514; and responding to social interactions, r = .346). See Table 3 for r- and p-values for all variables.

Table 3. Correlations between early social communication and social emotional development in the SCT group.

Early social communicative domains	r-value	p-value
Initiating Joint Attention	.264	.138
Initiating Behavioral Requests	.453	.008
Initiating Social Interactions	.269	.130
Responding to Joint Attention	.514	.002
Responding to Behavioral Requests	-.114	.528
Responding to Social Interactions	.346	.049

Note: SCT = Sex Chromosome Trisomy.

The role of specific karyotype (XXX, XXY, XYY)

In order to investigate whether specific karyotype-subtype (XXX, XXY, XYY) was predictive of social emotional abilities and early social communication, linear regressions with dummy coding were carried out with social functioning as dependent variables. No significant predictive effects of karyotype-subtype were found, see Table 4 for exact F- and p-values.

The role of recruitment strategy

Within the SCT group we tested whether recruitment strategy was predictive of social emotion abilities and early social communication. We used a linear regression with dummy coding for the three recruitment strategies (A: prospective follow-up,

B: information seeking parents, and C: clinically referred cases group), and social functioning as dependent variables. No significant predictive effects of recruitment strategy were found on the Bayley SE and for five subscales of the ESCS. Only for the subscale Responding to Social Interactions of the ESCS, a predictive effect of recruitment strategy was found, see Table 5 for exact *F*- and *p*-values.

Table 5. Regression models of predictive recruitment strategy effect on social functioning (*F* (df), *p*-value).

	Recruitment strategy (prospective follow-up, information seeking parents, and clinically referred cases)
ESCS	$n = 33$
Initiating Joint Attention	$F(2,30) = 1.52$, $p = .236$
Initiating Behavioral Requests	$F(2,30) = 0.53$, $p = .597$
Initiating Social Interactions	$F(2,30) = 0.44$, $p = .646$
Responding to Joint Attention	$F(2,30) = 0.98$, $p = .387$
Responding to Behavioral Requests	$F(2,30) = 0.83$, $p = .444$
Responding to Social Interactions	$F(2,30) = 3.77$, $p = .035$
Bayley SE	$n = 34$
Social emotional behavior	$F(2,31) = 1.04$, $p = .367$

Note: ESCS = Early Social Communication Scales.

Discussion

The aim of the current study is to investigate the early impact of SCT (XXX, XXY, XYY) on both responding and initiating early social communicative behaviors and parent-reported daily life social emotional functioning of young children, aged 12-24 months. On average, children with SCT show reduced frequency of eye contact in responding to social communication, and appeared to have vulnerabilities in early social communication and social emotional development. These findings were independent of global cognitive and language functioning. No karyotype specific differences (XXX, XXY, XYY) were found.

We used systematic behavior observations to explore social communication

behaviors in 1-year old children with SCT when they were actually exposed to social interactions in a structured play situation. These structured observations show that, on average, 12-24 months old children with SCT display lower frequency of eye contact in responding to social communication, as compared to their peers. Overall the systematic behavior observations indicate that 1-2 years old children with SCT have difficulties with early social communication, expressed on the domains of joint attention, non-verbal expressions of desires and/or beliefs, and reciprocal social interactions. These findings are in line with results on parental reports of domains of problem behavior operationalized with the Child Behavioral Checklist (Urbanus, Swaab, Tartaglia, Cordeiro & Van Rijn, 2020), showing that social emotional differences may already be present from the age of one year, and independent of karyotype (XXX, XXY and XYY).

Next, in exploring detailed aspects of early social communication in young children with SCT, it is found that children with SCT seem to have more difficulties in the way they respond to social initiatives of others, compared to how they initiate social communication. To illustrate, the ability of young children with SCT to initiate joint attention is intact, but they have difficulties with following the direction of gaze and gestures of others, i.e. responding to the invitations for joint attention of others. A similar pattern is found in the way young children in SCT are involved in reciprocal social interactions: young children with SCT are able to initiate interactions, but responding to others' invitations of social interactions is impaired. Difficulties with responding to social interactions are focused on vulnerabilities in making eye contact while responding to social interaction invites: although no overall difference between the SCT and control group was found on the domain of responding to social interactions, it was found that children with SCT make eye contact less often than their typically developing peers. These results suggest that 1-2 years old children with SCT do not deliberately use eye contact to respond to invitations for social interaction, but are capable of shaping a response behaviorally. With regard to the communication of desires and beliefs, young children with SCT have difficulties with both communicating their own desires and beliefs and to respond to desires of a social partner. Although speculative, the global finding that on average social communication is more affected in the way 1-2 years old children with SCT *respond* to social communication of others compared to how they *initiate* social communication is in line with a reported decreased attention to focus on key social information

such as faces and eyes (as for example found in children with SCT and adults with XXY; Bouw, Swaab, Tartaglia, Cordeiro & Van Rijn, 2021; Van Rijn, 2015). Existing evidence also show that children and adults with XXX and XXY have difficulties with understanding, following and labeling social cues of others as for example social gaze directions, and recognition of facial affects (Samango-Sprouse et al., 2018). From the age of four years old difficulties with generating adequate social behavioral responses to a social partner was reported in XXX, XXY and XYY (see for a review: Van Rijn, 2019). However, independent of the mechanisms that leads to difficulties with social communication as found in the current study (either difficulties with perceiving, processing or reacting to social information), the difficulties with social communication early in life of children with SCT may lead to difficulties with forming reciprocal social contact with others.

The results from the current study regarding the early impact of SCT on social emotional development are in line with this suggestion, as they show that social communication deficits in young children with SCT extend to broad social emotional functioning in daily life settings as reported by parents. It was found that the children with SCT had more difficulties showing some typical socio-emotionally behaviors, such as a limited ability to search proximity of the caregiver, and with showing imitations of familiar make-believe play and emotions in a back and forth way. In this study, the difference on social emotional development between children with SCT and their peers remain significant after controlling for general cognitive development. With regard to social emotional development, the proportion of children with SCT that scored in the borderline (38%) or extremely low range (6%) reveal that these social emotional impairments are present in a substantial subset of 1-year old children with SCT.

The age range of the participants in this study (12-24 months) encompasses a period of development where many milestones are achieved each month. Within the SCT group, positive associations were found between age and social emotional development and between age and some of the domains of social communication (i.e. Initiating Behavioral Requests, Responding to Joint Attention, Responding to Social Interactions) These results suggest an age related development of social emotional and social communicative abilities in older children with SCT. Further investigations are needed to study longitudinal pathways of individual children to

study early social developmental milestones even more in depth.

Even though social-emotional and communication vulnerabilities are found, our findings of intact ability to initiate joint attention and initiate social interaction tentatively suggest that on average 12-24 month old children with SCT have a motivation to spontaneously seek and share affective experiences with others. Earlier studies on parent-report social motivation of children with SCT from the age of four years found mixed results: depending on age and included karyotypes studied, no impact of SCT on social motivation was found in 4-18 years old with XXY or XYY by Cordeiro, Tartaglia, Roeltgen & Ross (2012), whereas two other studies did find an impact of SCT on social motivation in 9-18 years old with XXX or XXY (Van Rijn et al., 2014) and 6-21 years old with XXY (Tartaglia, Cordeiro, Howell, Wilson & Janusz, 2010). However, it should be noted that these studies utilized different parents report measures and had differences in sample ascertainment. Follow-up studies are needed to explore the developing pathways of motivation for social communication and underlying social cognitive and motivational mechanisms in young children with SCT from the first years of life into childhood. This is especially important in order to find targets of early/preventive support and intervention, based on the idea that early intact motivation for social contact should be preserved over the course of development.

The impact of SCT on social communication and social emotional development early in life as found in this study have implications for our understanding of brain-behavior pathways leading to these difficulties. The biological predisposition of SCT allows us to study early social development of a homogeneous group of children, that may serve as 'high risk' group when it comes to neurobehavioral social development. Raznahan et al. (2016) found that in a group of participants with Sex Chromosome Aneuploidies aged 5-25 year old, the X and Y chromosome congruently impact the functionality of cortical areas that support adaptive socio-emotional functioning and social communication (e.g. medial prefrontal cortex, anterior cingulate, and superior temporal sulcus). The difficulties we detect on the social domain in 12-24 month old children with SCT may reflect an impact of SCT on the maturation of an integrated social brain network already from infancy on, as these social difficulties are behavioral expressions of an impaired maturation of cortical brain networks that underlie social development (Johnson et al., 2005). The finding that there are no

differences between karyotype-subtypes (XXX, XXY, XYY) on social emotional and communicative difficulties is in line with a convergent influence of the extra X and Y chromosome on social brain maturation and associated social behavioral functioning. However, it was shown that the social behavioral profile in boys with 47,XYY is more vulnerable as compared to girls and boys with an extra X chromosome, which is illustrated by a higher risk for ASD (Cordeiro, Tartaglia, Roeltgen & Ross, 2012; Ross et al., 2012; Tartaglia et al., 2017). This more pronounced vulnerability in the XYY group was not found in the current study. However, our sample size in the XYY group was small. These findings call for more research into the nature of early social cognitive and -behavioral developmental pathways in boys with 47,XYY leading to the more pronounced reported social behavioral difficulties from school age on, as compared to boys and girls with an extra X chromosome.

From a developmental perspective, it is known that vulnerabilities in early social communication and emotional functioning have a high impact on further social (cognitive) development (Mundy & Newell, 2007). To illustrate, in responding to invitations of joint attention young children show their understanding of bids to engage in another's attention. A decreased tendency to respond to these invitations may contribute to an impaired development of learning to decode and reason about mental states of others (i.e. Theory of Mind; Sodian & Kristen-Antonow, 2015). Also, difficulties with social communication (e.g. less frequent eye contacts and difficulties with joint attention) early in life are reported in children with neurobehavioral disorders, such as Autism Spectrum Disorders (ASD; see for a review: Sivaraman, Virues-Ortega & Roeyers, 2020). Vulnerabilities for social emotional problems and difficulty with social communication early in life may thus be markers for difficulties in broad social functioning later on in life, and signs of potential co-morbid conditions such as ASD, which is shown to be significantly elevated in SCT as compared to the typical population (Van Rijn, 2019). In particular, the ability to response to joint attention has been frequently reported in the literature as an early marker for ASD (Mundy, 2017), and therefore also serves as an important target to monitor during early development in young children with SCT.

Deficits in early social communication may not only be associated with impairments in subsequent social development, but also with an altered language acquisition, speech delay and behavioral problems during early development (Pickard & Ingersoll,

2015; Schietecatte, Roeyers & Warreyn, 2012). It is found in non-clinical samples that early social skills are associated with school readiness and early school success (Ziv, 2013). Given the association of early social emotional and communication with impairments on subsequent development across domains, the outcomes of the current study may have considerable clinical implications. It is important to closely monitor patterns of social communication in young children from an early age on, as difficulties in early social communication (e.g. responding to joint attention, making eye contact) may be key indicators of compromised cross-domain development. Close monitoring of vulnerable children with SCT and if necessary early support and tailored intervention may positively influence social development through childhood.

The current study has both strengths and limitations. Strengths include the structured observations of social interactions, that allow us to measure observed social communicative behaviors of 1-year old children with SCT in interaction with a social partner. Second, the study sample consisted mainly (94%) of children that were prospectively studied after a prenatal diagnosis of SCT which suggest that our findings are highly representative for the group of diagnosed children. In this study, social outcomes were largely not dependent on recruitment strategy (i.e. prospective follow-up group, information seeking parents group, or clinically referred cases group), which suggests that on average our findings are representative for this group of diagnosed children. However, it should be taken into account that the sample sizes were small and vary between the recruitment groups. Similar, although no predictive effect of karyotype-subtype was found on social outcomes, it is important to consider the small sample sizes and the small number of participants in the XXX and XYY group which calls for a careful interpretation of the non-significant differences between karyotype-subtype. Additional studies with larger sample sizes are needed to investigate the individual X and Y chromosome influences on social functioning very early in life of children with SCT. Next, parents of children with SCT are aware of their child's diagnoses of SCT. As a consequence, they may rate the items of the Bayley SE questionnaire differently compared to parents of children in the control group, as parents of children with SCT are more or less aware what to expect in terms of their child's developmental outcome. This may have biased the outcome of parent reports.

The findings of this study deserve further investigation of the longitudinal effects of

early difficulties in social competence on social cognitive and behavioral outcomes and related neurodevelopmental psychopathology in children with SCT. These longitudinal effects will be further investigated in this population with prospective follow-up. As it was beyond the scope of this study to investigate the role of testosterone treatment in boys with 47,XXY, future studies with an applicable design (i.e. Randomized Controlled Trials) are needed to explore the influence of these variables in association with social behavioral functioning in young children with SCT.

Conclusion

In summary, the results of this study show that already very early in development, i.e. at the age of 1-year, children with SCT have vulnerabilities in social emotional functioning and the early ability to socially communicate with others. These results suggest an impact of SCT on social functioning very early in life, and indicate that social difficulties found later in life of individuals with SCT are anchored in early social brain development. The study of this genetic 'high risk' group, characterized by an extra X or Y chromosome may provide unique insights in the predictive value of early social communication deficits for neurobehavioral problems, learning impairments and mental health problems later in life. The findings of the current study advocate for close monitoring and early (preventive) support targeted at the earliest stages of social communication, with the aim to support social development of children with SCT from infancy on.

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CHAPTER 3

Early social behavior in young children with Sex Chromosome Trisomies (XXX, XXY, XYY): profiles of observed social interactions and social impairments associated with Autism Spectrum Disorder (ASD)

Bouw, N., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2022). Early Social Behavior in Young Children with Sex Chromosome Trisomies (XXX, XXY, XYY): Profiles of Observed Social Interactions and Social Impairments Associated with Autism Spectrum Disorder (ASD). *Journal of Autism and Developmental Disorders*, 1-14.

Abstract

Objective: Individuals with SCT (Sex Chromosome Trisomies; XXX, XXY, XYY) have an increased vulnerability for developing challenges on the social domain, given the convergent impact of the X and Y chromosome on brain networks that underly social adaptive development. This study aimed to investigate the early development of social interactive behavior in the context of varying social load, and social impairments associated with Autism Spectrum Disorder (ASD) symptomatology in young children with SCT.

Methods: The sample consisted of 105 children with SCT (range 1-7.5 years old), as well as 101 age-matched non-clinical controls. A structured behavior observation, the Interaction subtest of the Autism Screening Instrument for Educational Planning, third edition (ASIEP-3) was used to measure social interactive behavior. Social impairments were measured using the Social Responsiveness Scale, second edition (SRS-2). Recruitment and assessment took place in the Netherlands and in the United States.

Results: On average, young children with SCT showed less interactive behaviors and more social withdrawal, as compared to their control peers. These withdrawn behaviors were most evident in the high social load condition, and showed to be stable during early development. No differences between karyotypes were found for social interactive behavior. Social impairments were more prevalent in all karyotypes (XXX, XXY, XYY) from early age on, as compared to controls. 27.1% of the children with SCT had social impairments associated with ASD at a clinical level.

Conclusions: Already from a very early age on, SCT can be associated with increased risk for vulnerabilities in social interactions, and with increased levels of social impairments associated with ASD. These findings suggest that SCT impact the maturation of the social brain already from an early age, and stresses the importance of early routine monitoring and (preventive) support of early social development in young children with SCT.

Introduction

Approximately 1-650 to 1-1000 children is born with a Sex Chromosome Trisomy (SCT; Boyd et al., 2011). SCT, the presence of an extra X or Y chromosome, lead to the chromosomal patterns of 47,XXX in girls (Triple/Trisomy X), and 47,XXY (Klinefelter's syndrome) and 47,XYY (XYY syndrome) in boys. SCT has been associated with a mild physical phenotype shared across SCT conditions, such as mild facial characteristics, a tall stature, and low muscle tone (Tartaglia et al., 2020). Cognitive functioning in SCT is within normal limits, although somewhat lower than average, specifically with respect to the language domain (see for a review: Leggett et al., 2010). Specific effects of the extra X and Y chromosome on neurobehavioral development have been found in self-regulation and social adaptation (Van Rijn, 2019; Urbanus et al., 2020; Tartaglia et al., 2020). SCT is consequently associated with increased risk for symptoms of social difficulties and Autism Spectrum Disorders (Van Rijn, 2019).

To understand the pathways to impairment of social adaptation it is important to be aware that the presence of an additional X or Y chromosome is known to convergently impact the maturation of brain functions and networks involved in social adaptive cognitive and behavioral development (Hong & Reiss, 2014; Raznahan et al., 2016). Social cognition involves the abilities that enable us to understand social information and to interact with the social environment (Beauchamp & Anderson, 2010), and are central to interpersonal communication, to the development and maintenance of satisfying relationships with others (Rao et al., 2008), and are associated with quality of life (De Vries & Geurts, 2015). Since social adaptive development is anchored in early brain maturation and because the first years of life are important for rapid maturation and specialization of the social brain network, this period may mark a key period for the development of appropriate processing of social information, allowing successful social interaction with others (Grossmann & Johnson, 2007). It is therefore especially important to investigate the impact of SCT on social cognitive abilities and social behavior during the early stages of childhood, to identify early markers of an 'at risk' social development. The current study was designed to provide insights into that, and focuses on the early impact of SCT on social interaction behaviors and risk on social impairments related to ASD in children aged 1- 7.5 years.

Although individuals with SCT have unique and varying developmental profiles, there

is sufficient evidence that, on average, the social behavioral presentation of SCT is characterized by challenges in social functioning. School-aged children, adolescents and adults with SCT may show shy, withdrawn and anxious social behavior, as well as difficulty with forming personal relationships, and with taking initiative in social contact (Bender et al., 1999; Otter et al., 2010; Ross et al., 2012; Van Rijn et al., 2014). The outcomes of these studies suggest that individuals with SCT from school age on are at risk of experiencing difficulties in social adaptive functioning. However, research on social development before the age of six years is extremely limited. In order to sensitively and objectively explore the impact of SCT on social functioning early in development, we studied social interaction behaviors of young children with SCT during structured behavior observations. Increasing social interaction skills are necessary for developing the capacity to deal with more complex social information with more social information and a higher pressure to react in a social adaptive way (Soto-Icaza et al., 2015). We were therefore interested whether and how young children with SCT shape their social interaction behavior under varying levels of social load during social interactions. We manipulated the social load factor during the social interaction observation from social interactions with a low social load, in which the adult provided a play example for the child to follow but did not maintain the interaction, to a social interaction with no social load in which the adult remains passive in which it was up to the child to initiate and continue the interaction, and finally a social interaction with a high social load in which the adult gave the child directions and cues in a stable and high frequency which could lead to some minor stress in the child (Krug & Almond, 2008).

The severity of the impact of SCT on social development is illustrated by reports of increased risk for social impairments that are associated with ASD symptomatology in SCT. ASD is a clinical classification of neurobehavioral problems resulting in persistent deficits in social interaction and communication, and by repetitive behaviors and restricted interests (DSM; American Psychiatric Association, 2013). Compared to a worldwide prevalence rate of ASD of 0.6% in the general population (Elsabbagh et al., 2012), prevalence of ASD has been shown to be higher in SCT. On average across studies, depending on the ascertainment methods, diagnostic measurements and criteria used, 15% (range 10.8-20%) of individuals with 47,XXX meet full ASD criteria; 18% (range 10-27%) of individuals with 47,XXY and 30% (range 19-43%) of individuals with 47,XYY (see for a review: Van Rijn, 2019). Rather

than investigating the risk for ASD symptomatology as a categorical phenomenon, previous studies have also explored the impact of SCT on a range of social behavioral symptoms associated with ASD. These studies found high levels of ASD symptomology from school age onwards, with a relative strength with regard to social awareness and social motivation for boys and girls with an extra X chromosome and a strength in social motivation for boys with 47,XYY (Tartaglia et al., 2010; Van Rijn et al., 2014; Cordeiro et al., 2012; Wilson et al., 2019). However, these studies had broad age ranges from childhood to early adulthood, and did not investigate the impact of SCT on social impairments very early in life. To explore the extent to which early social vulnerabilities reflect high levels of symptoms that may belong to ASD, the present study investigates the impact of SCT on social impairments in the domains of social awareness, social cognition, social communication, social motivation and if there are restricted interests and repetitive behaviors during the first years of life.

Learning more about the early social development of children with SCT will shed light on early neurocognitive and neurobehavioral pathways to social challenges and related psychopathology later in life of children, adolescents and adults with SCT. Urgency for this knowledge is stressed by the increasing number of children diagnosed with SCT, as a result of advanced technology to screen for genetic variations before birth (i.e. NIPT; Samango-Sprouse et al., 2017; Tartaglia et al., 2020). This growing group of prenatally diagnosed children with SCT also provides us with the unique opportunity to prospectively explore neurobehavioral profiles of a genetic at risk population, even before the behavioral phenotype of the genetic condition is revealed. That is to say, exploring early neurobehavioral profiles helps us to understand developmental pathways leading up to behavioral symptoms of SCT. In the literature, vulnerabilities in individuals with SCT are predominantly described in terms of global cognitive (i.e. intellectual functioning) and language deficits (Boada et al., 2009; Leggett et al., 2010; Ross et al., 2009), although recent studies also explored the impact of SCT on other neurocognitive outcomes, such as social cognition and executive functioning (see for reviews of findings in school age children and adolescents: Van Rijn, 2019; Urbanus et al., 2020). Although speculative, difficulties in coping with the social environment may not only be the result of a lower general understanding of the environment, but also lower levels of abilities to interact with the environment in terms of language and communication. A socially vulnerable developmental course may also be related to specific early impacts of SCT on social

(cognitive) abilities such as being aware of social information, understanding social information, and shaping social interaction behaviors such as smiling, eye contact and imitation. Support for this hypothesis comes from recent studies that found an early impact of SCT on social cognition (Bouw et al., 2021), and social behavioral problems (Urbanus et al., 2020). We therefore studied the role of global cognitive and language abilities in social adaptive behavior of children with SCT.

Take together, the current study aims to explore the early impact of SCT on profiles of social interaction behaviors under different levels of social load, and parent-reported social impairments associated with ASD in young children aged 1-7 years old, with regard to the type of vulnerability, the age dynamics and clinical severity of these behaviors. Second to these main research questions, we investigate the role of cognitive and language development on social outcomes, and to what degree the specific karyotypes (XXX vs. XXY vs. XYY) vary in risk for social vulnerabilities. Based on the relevance of the extra X and Y chromosome on brain networks that underlie the development of social adaptive behavior, and reported vulnerability for social difficulties in individuals with SCT, we hypothesized that on average young children with SCT might show less well developed social interaction abilities and elevated social impairments associated with ASD, as compared to a control sample. We also hypothesized a specific effect of SCT on social functioning, that is to say that the impact of SCT on social functioning is independent of global cognitive and language development.

Methods

Participants

The present study is part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study - Leiden, The Netherlands), which includes children with SCT and nonclinical controls aged 1-7.5 years. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. A group of 105 children with SCT (range 1-7.5 years old; M_{age}= 3.66, SD = 1.94) was included in this study, as well as a population-based sample of 101 children (44 boys; M_{age}= 3.61, SD = 1.63). Mean age did not differ between groups ($t(204)$ = 0.21, p = .837). The SCT group consisted of 33 girls with 47,XXX (31.4%), 50 boys with 47,XXY (47.6%) and 22 boys with 47,XYY (21.0%). Age did not differ between

karyotypes (F (2,102) = 2.81, p = .065). Recruitment and assessment took place at two sites: the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, 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 in cooperation with the clinical genetics departments (from The Netherlands and Colorado, USA), as well as through patient-advocacy groups and social media postings. The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping. Seventy-one children (67.6%) were diagnosed prenatally (20 girls with XXX, 36 boys with XXY, 15 boys with XYY), and 34 children (32.4%) postnatally (13 girls with XXX, 14 boys with XXY, 7 boys with XYY). As ASD cannot be diagnosed prior to the age of 2, diagnosis status was available for 68% of the sample. Within this group, three parents reported that their child received a clinical diagnosis of ASD (1 boy with XXY, 2 boys with XYY).

For the SCT group, recruitment strategy was assessed, and three subgroups were identified: (1) 'active prospective follow-up', which included families who were actively followed after prenatal diagnosis (51.4% 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 (29.5% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development (19.0% of the SCT group). Twenty-four out of 50 boys with 47,XXY had received testosterone treatment (48.0%).

Control children were recruited from the western part of The Netherlands, and approached with information brochures about the study. All participants (child and parents) were Dutch or English speaking, had normal or corrected-to-normal vision, and did not have a history of traumatic brain injury. 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 ~1 in 1000, the risk of having one or more children with SCT in the control group was considered minimal and acceptable.

Measurements and Instruments

Structured observations of social interactions: ASIEP-3. The Interaction subtest of the Autism Screening Instrument for Educational Planning - Third Edition (ASIEP-3; Krug & Almond, 2008) was administered, in order to measure social interaction behaviors including spontaneous social responses, acknowledgement of direct requests, and the ability of the child to socially interact with an examiner under different conditions of external social load. The ASIEP-Interaction subtest is a time structured and standardized play setting, and consists of three different environmental conditions of four minutes each that were always administrated in the same order: 1) the active modeling condition: basic inviting parallel play modelled by the examiner, defined as the low social load condition, 2) the passive/no interaction condition: the withdrawal of engagement and attention by the examiner, defined as the no social load condition, and 3) the direct cues condition: the examiner gives specific cues and directions to the child, defined as the high social load condition. The examiner used age appropriate toys and activities during the play situation, as well as language appropriate for the skill level of the child. See Cordeiro et al. (2012) for additional descriptions of the interaction assessment, and Figure 1 for an overview of the three conditions and task administration descriptions.

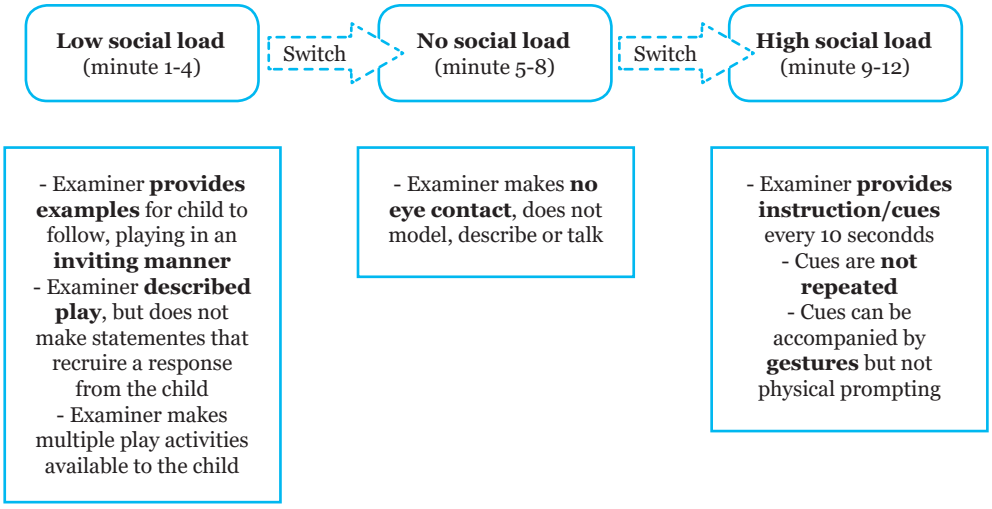


Figure 1. Administration of ASIEP-3, Interaction Assessment. Conditions of social load: administration and examples of statements (adapted from Cordeiro et al., 2020).

The ASIEP was video recorded and scored afterwards by trained independent raters, based on the procedures described by Krug & Almond (2008). Raters were not involved in the assessment, and blind to the child's group membership and karyotype. The videos were scored at 10-second intervals, and the observed behaviors were scored into one of four behavior codes: 1) Interaction, e.g. the child responds, initiates, touches or complies, 2) Constructive Independent Play, e.g. independent play without social interaction, 3) No Response, e.g. no observable behavior or response of the child, or self-stimulation and self-abuse, or 4) Aggressive behavior, e.g. tantrums, hits, cries, bites, etc.. The codes are summed yielding counts for each of these four behavior codes, within the three conditions (i.e. absent, low and high social load), and an overall total score (possible range 0-48).

Social impairments associated with Autism Spectrum Disorder (ASD): SRS-2. In order to measure ASD symptomology, the primary parent of the child completed the Social Responsiveness Scale – Second Edition (SRS-2; Constantino & Gruber, 2012). The SRS is a 65 item parent-report questionnaire, designed to quantify ASD related social impairments. Dependent on the age of the child, two different versions of the SRS-2 were administrated to the primary caregiver of the child: the SRS - Preschooler version (children aged 3-4 years old) and the SRS - School age version (children aged 4-7.5 years old). Examples of items are: 'Is able to understand the meaning of other people's tone of voice and facial expressions' and 'Responds appropriately to mood changes in others (for example, when a friend's or playmate's mood changes from happy to sad)'. The parent rated the behavior of the child over the past six months on each item using a 4-point Likert scale ranging from 1 'not true' to 4 'almost always true', yielding a raw total score and gender-normed T-score on five subdomains of ASD symptoms (social awareness, social cognition, social communication, social motivation and restricted interests or repetitive behavior). Higher raw and T-scores indicate parental report of a higher and more severe presence of ASD symptoms. In addition to average behavioral outcomes on the five subdomains, we were also interested in percentages of children with SCT that had scores above clinical cut-off, indicating clinical risk of ASD. T-scores were used to calculate risk of ASD symptoms that are clinically relevant and associated with a clinical diagnosis of ASD. T-scores between 65 and 75 correspond to a 'moderate' range of severity, and scores of 76 and higher are in the 'severe' range. The SRS-2 has strong internal consistency (Constantino & Gruber, 2012), and extensive proof of validity (Bruni, 2014).

Global level of cognitive and language development. In order to measure global level of intelligence, receptive and expressive language, developmental age appropriate instruments were used. The Bayley - Third Edition (cognitive, receptive language and expressive language scale; Bayley, 2006) was administered to 1-2 year old children. In the older children four subtests of the Wechsler Preschool and Primary Scales of Intelligence - Third Edition (WPPSI-III; Wechsler, 2002) were used to estimate global level of intelligence (children aged 3 years: Block Design, Receptive Vocabulary, Information, Object Assembly; children aged 4 years and older: Block Design, Matrix Reasoning, Vocabulary, and Similarities). For children aged 4 years and older, Total IQ estimates were calculated based on this short form version of the WPPSI-III (Hurks et al., 2016). The Peabody Picture Vocabulary Test - Third Edition (PPVT-III; Dunn & Dunn, 1997) was used to measure receptive language level in children aged 3 years and older. To assess expressive language skills, the Clinical Evaluation of Language Fundamentals - Preschool, Second Edition, was administrated to children of 3 years and older (CELF-Preschool; Wiig et al., 2004).

Ethical approval and informed consent

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. Signed informed consent was obtained from the parents/guardians of all participating children, according to the declaration of Helsinki.

Study procedures

Assessment took place at various sites (Colorado (USA) and The Netherlands) 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 for 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). Administration of the WPPSI-III, CELF-Preschool and PPVT-III was performed seated on a table by trained child psychologists in the Dutch or English language, depending on the first language of the child. The SRS-2 questionnaire was filled in by the primary caregiver of the child, either in Dutch or English. Administration of the ASIEP always took

place after fixed amount of interaction time with the child before starting the test in order to prevent familiarity differences to interfere with the test scores.

Statistical analyses

Statistical Package for the Social Sciences (SPSS, version 25) was used for statistical analyses. Independent t-tests were used to test for differences between research sites. Repeated Measures ANOVAs were used to study differences in profiles of social interaction between the SCT and control group, in which the Greenhouse-Geisser correction was used if the assumption of sphericity was violated. To test for differences between the SCT and control group for social impairments, a MANOVA was carried out. Moderating effects of age on social interaction behavior and social impairments were tested with PROCESS analyses (Hayes, 2017). Pearson's correlation were used to assess the association between social behavioral outcomes and cognitive abilities. When significant correlations were found, the cognitive parameter was added to the analyses as covariate (RM MANCOVA/MANCOVA). Two separate t-tests were used to measure SCT vs. control differences of social impairments in average and below-average IQ groups. Differences on social behavioral outcomes between karyotypes (XXX, XXY, XYY) and recruitment bias groups (active follow-up, information-seeking parents, clinically referred cases) were assessed and accounting for the effect of age with ANCOVAs. Statistical significance was set at $p < .05$ a priori. Statistical analyses were performed one-tailed (SCT vs. control) or two-tailed (moderating age effect, influence of karyotype, recruitment bias). Effect sizes were calculated with partial η^2.

Results

Comparison between research sites

No differences between research sites (The Netherlands, USA) were found for total score on the ASIEP-3 (t (87) = -1.50, p=.138), and for total score on the SRS-2 (t (68) = -0.38, p=.707). Therefore, all SCT data were collapsed across sites.

Structured observations of Social Interaction

Data quality. The ASIEP-3 was successfully administrated and completed by 188 children enrolled in the study. 18 children were not able to complete the ASIEP

administration, mainly due to non-compliance or unstandardized administration. Interrater reliability was calculated based on a randomly selected subsample of 10 participants, and showed an intraclass correlation coefficient (ICC) of .86 - .89 (for the ASIEP behavior codes collapsed together), which is considered excellent reliability (Cicchetti & Sparrow, 1981).

Social interaction behavior in SCT vs. control. Over all ages, a significant difference between the SCT and control group was found for social interaction behavior, independent of social load condition (F (2.03, 376.97) = 9.17, p <.001, η_p^2= .05). Pairwise comparisons revealed less Interaction behavior and more Constructive Independent Play in the SCT group as compared to control children, with medium effect sizes. No differences were found for No Response and Aggressive behaviors (see Table 1).

Table 1. Structured observations of social interactions in the SCT and control group: behavioral outcomes.

	N	Missing	SCT		Control		SCT vs. control		
			Min-Max	*M (SD)*	Min-Max	*M (SD)*	*p*-value	Group differences	Effect-size (η_p^2)
Social interaction behavior	188	18							
Interaction			0-37	17.92 (7.86)	6-43	0-13	<.001	SCT < control	.07
Constructive Independent Play			0-41	21.66 (8.50)	1-39	17.24 (8.34)	<.001	SCT > control	.07
No response			0-41	.21 (8.69)	0-34	7.90 (7.19)	.786		
Aggression			0-4	.09 (0.47)	0-13	.22 (1.38)	.393		

Note: SCT = Sex Chromosome Trisomy; ASIEP =Autism Screening Instrument for Education Planning.

To investigate the conditional effect of social load (no social load; low social load; high social load) on social interaction behavior between the SCT and control group, a RM ANOVA was used. A significant overall difference between the SCT and control group was found for social interaction behavior within the different conditions, $F(3.98, 775.83) = 7.90$, $p < .001$, $\eta_p^2 = .04$. In the no social load condition, as compared to controls the SCT group showed lower Interaction behaviors (p=.001), and a trend toward significance to higher Constructive Independent Play (p=.061). In the low social load condition, as compared to controls the SCT group showed higher Constructive Independent Play (p=.006). Lastly, in the high social load condition, as compared to controls, the SCT group showed lower Interaction behaviors (p <.001), higher Constructive Independent Play (p=.015) and lower No Response scores (p <.001; see Figure 2).

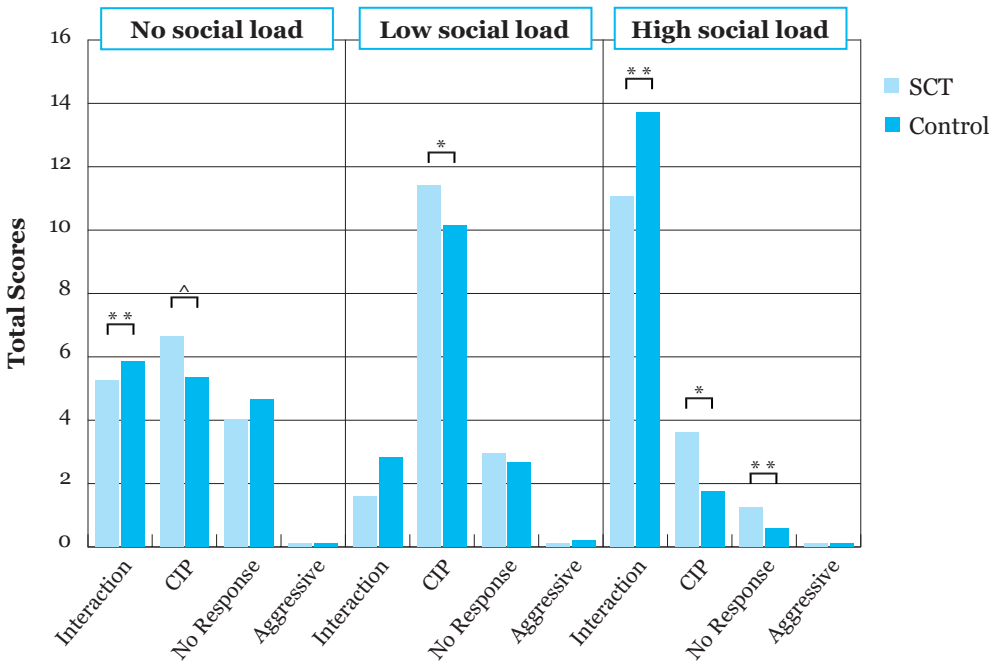
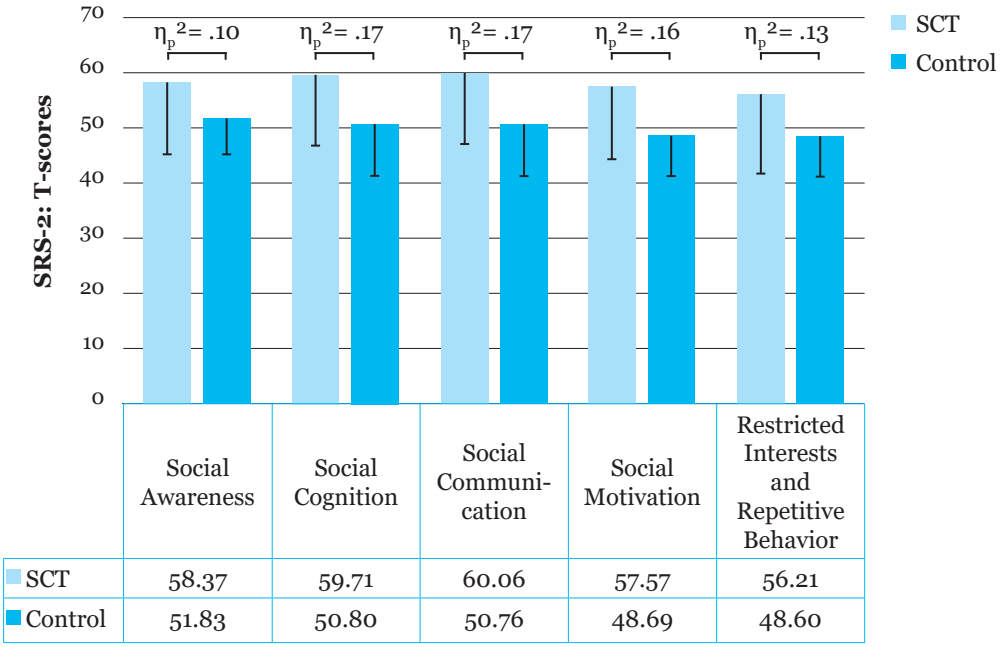


Figure 2. Social interaction behaviors (Interaction, CIP, No Response, Aggressive) under three conditions (no social load / low social load / high social load) in the SCT and control group.

Note: CIP = Constructive Independent Play; * = p <.05; ** = p <.001; ^ = trend toward significance (p <.10).

Social impairments associated with Autism Spectrum Disorders

Social impairments in SCT vs. controls. Scores on the SRS differed between the SCT and control group for all SRS subscales (F (5,134) = 11.87, p <.001, η_p^2= .31). These results indicate that children with SCT have elevated social impairments as compared to their peers, on the domains of social awareness (SCT: M = 9.13, SD = 3.41; control: M = 7.34, SD = 2.58), social cognition (SCT: M = 10.99, SD = 5.30; control: M = 5.64, SD = 3.29), social communication (SCT: M = 18.44, SD = 9.28; control: M = 9.31, SD = 5.42), social motivation (SCT: M = 9.59, SD = 5.83; control: M = 5.13, SD = 2.97), and restricted interests and repetitive behaviors (SCT: M = 6.99, SD = 5.68; control: M = 2.93, SD = 2.84), with medium to large effect sizes. When evaluating total scores normalized for age and gender 72.9% of the SCT group scored in the average range, and 27.1% showed elevated ASD symptoms that are clinically significant: 15.7% of the children with SCT scored in the moderate range (T-score > 65 and < 75), 11.4% scored in the severe range (T-score < 75). See Figure 3 for T-scores in the SCT and control group on domains of the SRS.



	Social Awareness	Social Cognition	Social Communi-cation	Social Motivation	Restricted Interests and Repetitive Behavior
SCT	58.37	59.71	60.06	57.57	56.21
Control	51.83	50.80	50.76	48.69	48.60

Figure 3. Social impairments associated with Autism Spectrum Disorder (ASD) in the SCT and control group (Mean T-scores).

Note: η_p^2= effect size; ⟷ = Standard Deviation (only lower bar depicted).

Developmental effects on early social behavior

In order to investigate the moderating effect of age on differences between the SCT and control group PROCESS analyses were carried out. Because aggressive behavior was almost non-existent in both the SCT and the control group during the ASIEP, and the distribution of aggression was insufficient, aggressive behavior was not included in the analyses. No moderating effects of age were found on social interaction behavior. See Table 2 for exact B, *t*- and *p*-values of the PROCESS models. Regarding social impairments, a moderating effect of age was found for social awareness (p=.028), indicating that the difference between children with SCT and control children was larger in older ages (see Figure 4).

Table 2. PROCESS models of the moderating effect of age (group x age) on social interaction behavior and social impairments in the SCT and control group.

Social Interaction Behavior (*N* = 188; age 1-7.5 years)		**B**	**95% CI**	t_{age}	p_{age}
No social load	Interaction	.31	-.15, .77	1.32	.188
	CIP	.33	-.42, 1.07	.87	.384
	No Response	-.33	-.95, .28	-1.07	.287
Low social load	Interaction	.40	-.25, 1.04	1.22	.225
	CIP	-.06	-.69, .56	-0.20	.838
	No Response	-.24	-.85, .38	-0.76	.450
High social load	Interaction	-.16	-.74, .43	-0.53	.595
	CIP	-.02	-.50, .46	-0.09	.929
	No Response	.11	-.21, .42	0.66	.507
Social Impairments (*N* = 140; age 3-7.5 years)					
Social Awareness		-1.02	-1.93, -.11	-2.22	.028
Social Cognition		-.98	-2.31, .36	-1.45	.150
Social Communication		-2.01	-4.30, .29	.09	.086
Social Motivation		-.60	-2.00, .81	-0.84	.403
Restricted Interests and Repetitive Behavior		-.91	-2.26, .45	-0.35	.188

Note: CIP = Constructive Independent Play.

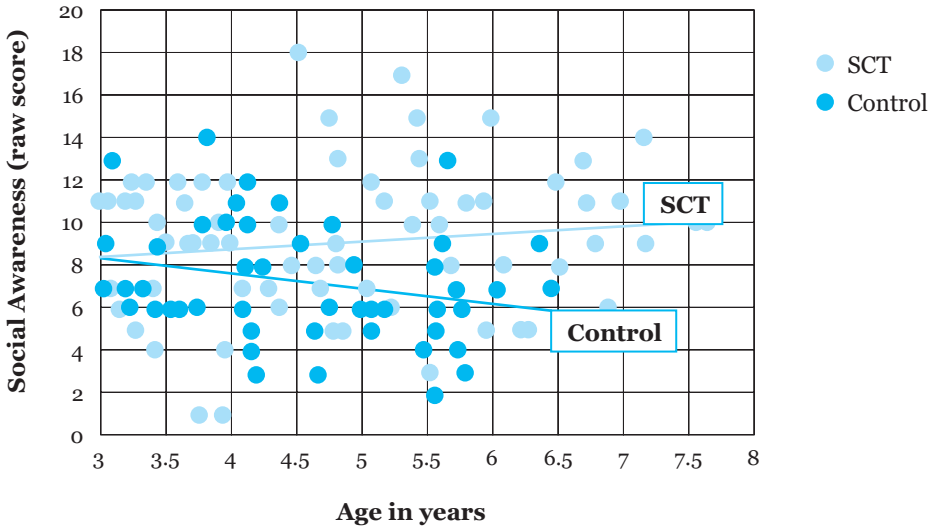


Figure 4. Moderating effect of age on social awareness; higher scores indicate difficulties in social awareness.

Note: SCT = Sex Chromosome Trisomie.

The role of global cognitive and language level in early social behavior

Structured observations of social interactions. Behavioral outcomes on the ASIEP-3 (Interaction, Constructive Independent Play, No Response, Aggressive) were not correlated with global cognitive level and receptive language skills. However, total Interaction scores were positively correlated with expressive language skills (r =.142, p = .048). Therefore, expressive language skills were added as covariate in the group analyses. The overall differences between the SCT and control group remained significant, even when level of expressive language was added as covariate in the analysis, F (2.02, 659.21) = 6.97, p <.001, η_p^2= .04. See Table 3 for descriptive statistics of global cognitive level, receptive and expressive language skills in the SCT and control group.

Table 3. Descriptive statistics in the SCT and control group: cognitive and language skills.

	Age group	*N*	Missing	SCT	Control	SCT vs. control		
				M (SD)	*M (SD)*	*p*-value	Group differences	Effect-size (η_p^2)
Cognitive development standard score; Bayley-III	1-2 years	33 SCT 30 control	0	99.55 (13.60)	99.71 (13.98)	.962		
Receptive language development scaled score; Bayley-III	1-2 years	33 SCT 30 control	0	9.24 (2.87)	12.23 (2.57)	<.001	SCT < control	.24
Expressive language development scaled score; Bayley-III	1-2 years	33 SCT 30 control	0	9.33 (3.20)	11.45 (2.85)	.007	SCT < control	.11
Total IQ standard score; WPPSI-III	3-7 years	64 SCT 71 control	5	95.28 (19.70)	108.24 (13.85)	<.001	SCT < control	.13
Receptive language standard score; PPVT-III	3-7 years	64 SCT 71 control	6	99.70 (15.01)	108.67 (12.44)	<.001	SCT < control	.10
Expressive language scaled score; CELF-Preschool	3-7 years	64 SCT 71 control	4	8.18 (3.05)	11.38 (2.55)	<.001	SCT < control	.25

Note: SCT = Sex Chromosome Trisomy.

Social impairments. Total scores on the SRS were negatively correlated with global cognitive functioning (r = -.520, p <.001) and expressive language skills (r = -.490, p <.001), but were not correlated with receptive language skills. Therefore, global cognitive functioning and expressive language skills were added as covariate in the group difference analyses. The difference on social impairments between the SCT and control group remained significant, even when global cognitive functioning and expressive language skills were added as covariates, F (5,125) = 4.19, p=.001, η_p^2= .14.

In order to investigate whether social impairments were more pronounced in children with SCT with a below-average IQ, Total IQ is categorized into two groups (IQ<84: below average; IQ>85: average). The distribution of karyotypes (XXX, XXY, XYY) was similar between the two IQ groups (χ^2 (2) = .29, p=.864). Two separate t-tests were carried out to investigate differences in social impairments between SCT and control children in both IQ-groups. In the average IQ-group, we found differences between the SCT (M = 49.70, SD = 21.10), and control children (M = 30.18, SD = 13,47; t (71,97)=5.59, p <.001) with a large effect size (Cohens' d = 1.10). Similar, in the below average IQ-group, we found differences between the SCT (M = 67.67, SD = 30.32), and control children (M = 34.33, SD = 5.13; t (18,66)=4.31, p <.001), with a large effect size (Cohens' d = 1.53). Based on these statistical tests and comparison of the effect sizes, these results indicate that both SCT children with average IQ as well as the SCT children with below average IQ have significantly increased social impairments, with relatively more severe social impairments in the below average IQ-group.

Karyotype differences within the SCT group

MANCOVAs were carried out in order to measure differences between the various karyotypes on social interaction behaviors and social impairments, accounting for the effect of age. For social interaction behavior, no differences between karyotypes were found, F(8,166) = 0.81, p=.597. A significant difference between karyotypes was found for social impairments, when accounting for the effect of age (F(10,126) = 2.37, p=.013, η_p^2= .16). Across karyotypes, the XYY subgroup showed more pronounced social impairments in the domains of social cognition, social communication, and restricted interests and repetitive behavior, with medium to large effect sizes. Social

awareness and motivation were similar across the three karyotypes. See Table 4 for exact *M*, *SDs*, *p*-values and effect sizes.

Table 4. Differences in total social interaction behavior and social impairments across karyotypes (*M, SD*).

	XXX	XXY	XYY	*p*-value	Group differences	Effect size (η_p^2)
Social Interaction	*n* = 30	*n* = 44	*n* = 15			
Interaction	19.93 (7.24)	17.16 (7.29)	16.13 (10.12)	.478		
Constructive Independent Play	20.00 (8.00)	22.93 (8.83)	21.27 (8.41)	.259		
No Response	7.93 (9.17)	7.73 (7.53)	10.20 (11.02)	.362		
Aggressive	0.00 (0.00)	0.11 (0.62)	0.20 (0.41)	.521		
Social Impairments	*n* = 27	*n* = 29	*n* = 14			
Social Awareness	9.26 (3.15)	8.45 (3.28)	10.29 (4.05)	.300		
Social Cognition	11.78 (4.91)	9.10 (4.26)	13.36 (6.80)	.037	XYY > XXY	.10
Social Communication	18.78 (7.90)	14.55 (5.75)	25.86 (12.94)	.001	XYY > XXX/XXY	.20
Social Motivation	10.70 (6.47)	8.03 (3.58)	10.64 (7.73)	.222		
Restricted Interests and Repetitive Behavior	7.26 (4.64)	5.07 (4.18)	10.43 (8.35)	.019	XYY > XXY	.11

Note: Higher scores on domains of Social Impairments indicate more impairments.

Recruitment bias within the SCT group

Within the SCT group we tested with ANOVAs for differences on total scores between the three recruitment groups (prospective follow-up after prenatal diagnosis / information seeking parents / clinically referred cases), accounting for the effect of age. There were no significant differences for total social interaction behavior and social impairments associated with ASD, indicating that how children with SCT enrolled in the study was not related to their outcomes on social interaction behavior and social impairments. See Table 5 for exact *M, SDs* and *p*-values.

Table 5. Differences in total social interaction scores and social impairments across recruitment groups *(M, SD)*.

	Prospective follow-up	Information seeking parents	Clinically referred cases	*p*-value
Social Interaction	*n* = 49	*n* = 22	*n* = 18	
Total raw score	37.33 (14.23)	40.95 (16.09)	37.67 (12.36)	.426
Social Impairments	*n* = 30	*n* = 22	*n* = 18	
Total raw score	51.00 (24.58)	56.55 (26.20)	60.83 (25.20)	.414

Discussion

The current study adds to our understanding of the impact of SCT (XXX, XXY, XYY) on early social adaptive development in children aged 1 to 7.5 years. We evaluated profiles of social interactions in young children with SCT during structured behavior observations of a play situation, and vulnerability for social impairments that are associated with Autism Spectrum Disorder (ASD), based on daily life behaviors in a relatively large international sample.

Already early in life, an impact of SCT was found on the development of social interaction abilities: during a standardized behavior observation of a play situation, young children with SCT between the age of 1 and 7.5 years displayed on average less instances of social engagement as compared to their age matched peers, i.e. they showed less interaction behavior and more independent play. To illustrate, young children with SCT show a tendency to initiate and/or maintain less interactions, less imitations and mimics of play models, and to use less gestures and signs during communication, as compared to their age related peers. Also, young children with SCT more frequently demonstrate social withdrawal from interaction as evidenced by solo play. Aggressive or negative behaviors were almost non-existent in our study sample.

Interestingly, social interaction and withdrawn behavior differed as a function of social load: young children with SCT showed less social interactions when social load was absent, and more social withdrawal when social load was high, as compared to their peers. Social impairments were most pronounced when the level of social load was high, as children with SCT show less interactions and more severe social withdrawal (i.e. no observable or self-stimulating behavior) in the high social

load condition. These findings suggest that social input and demands from the environment are conditional for the formation of social behavior in interaction with the social environment, as the results show that children with SCT may be able to shape social behavior when the environment provides little social input, but that they have difficulties with actively coping with varying levels of environmental social load and with responding to complex social information (i.e. information with a high social load). When extending the analysis to control for the potential impact of cognitive and language abilities on social behavior during high social load, these patterns remained significant.

These results fit with earlier studies that investigate the impact of the extra X and Y chromosome on social phenotypes in older age groups, showing that SCT on average is associated with shyness, social withdrawal, difficulties in peer-relationships, reduced social assertiveness, and communication difficulties (Bender et al., 1999; Geschwind & Dykens, 2004; Otter et al., 2010; Van Rijn et al., 2014), and reported increased rick for mild symptoms of social anxiety (Van Rijn et al, 2014; Wilson et al., 2019). Our findings add to the existing knowledge that an impact of SCT on social development can already be found during the first years of life, a developmental period in which social adaptive behavior rapidly develops as a function of brain maturation (Soto-Icaza et al., 2015), and that social abilities are dependent on the complexity of social information. Social interaction vulnerabilities showed to be independent of age and karyotype (XXX, XXY, XYY), which may suggest a stable impact of SCT during early development. As fewer positive social experiences and more social avoidance during early life can lead to less opportunities to develop social adaptive behavior (Beesdo et al., 2009), it is important to monitor and (preventively) support early social cognitive and behavioral development of children with SCT.

The current study showed that children with SCT are better able to attune their social behavior in interactions if social load is low. It may be that children with SCT have difficulty with selecting sensory information from their environment that in turn drives the challenges they face with producing social adaptive behavioral responses. It is known that sensory processing plays a major role in vulnerabilities on the social domain in children with neurobehavioral developmental conditions, such as Attention-Deficit/Hyperactivity Disorder and Autism Spectrum Disorder, suggesting that difficulties with processing sensory information play an important

role in impairments in self-regulation and social adaptation (Cheung & Siu, 2009; Little et al., 2018; Sanz-Cervera et al., 2017). Basic sensory stimuli processing is fundamental in gaining access to social information from the environment and therefore in further shaping social behavior (Dellapiazza et al., 2021). Although difficulties in sensory processing are reported in individuals with SCT in clinical single case reports (Tartaglia et al., 2015), and the suggestion has been made earlier that rapid processing in auditory and other sensory modalities underlie language difficulties in adult men with 47,XXY (Geschwind et al., 2000), sensory profiles of individuals with SCT were not studied so far. It is therefore important to investigate the impact of SCT on profiles of sensory information processing on different sensory modalities, as for example on auditory and visual aspects of environmental stimuli.

The observed deficits in structured social interactions showed to be extend to impairments in daily life social behavior as reported by their parents, as we found that young children with SCT are at increased risk for social impairments associated with ASD. Within the SCT group, 27.1% had total social impairment scores in the clinical range (15.7% in the moderate clinical range and 11.4% in the severe clinical range). These findings are in line with earlier studies that investigated social impairments in individuals with SCT with broad age range groups from school age into adolescence (Cordeiro et al., 2012, Tartaglia et al, 2010, Van Rijn et al., 2014, Wilson et al., 2019). The current study adds to the existing literature by showing an impact of the extra X and Y chromosome on ASD symptomatology that is present from a very young age. We found that in a small age range sample (3-7 years), social impairments already arise early in life, and are relatively stable during early development.

More specifically, across all karyotypes we found increased risk for significantly elevated social impairments for all the measured dimensions: social awareness, social cognition, social communication and social motivation. Young children with SCT also showed significantly more restricted interests and repetitive behaviors as compared to their age matched peers. When comparing overall effect sizes of the SCT vs. control differences on domains of social impairments, social awareness was an area of relative strength, including abilities such as being aware of certain social cues as for example facial expressions and body language. Earlier studies investigating the impact of SCT on social impairments found comparable effects: Tartaglia et al. (2010) found a relative strength in social awareness in boys with XXY, and Cordeiro et

al. (2012) found mean scores for social motivation just above the normal range cutoff in boys with XXY and XYY. These results may suggest that children with SCT have relatively intact social awareness, which may be different from children with ASD, a reasoning that has already been suggested by Van Rijn et al. (2014) and Wilson et al. (2019). These findings are in line with the results of the structured play observations in the current study with respect to social interaction behavior, showing that level of social input and demands from the environment indicates specific types of social deficits, and that social interaction behavior seems to be less affected when the social load from the environment was low. These results may suggest that young children with SCT are aware of their social environment, but are not able to adequately shape their behavior during social interactions.

However, in our sample with an age range of 3-7 years, we found age variability for impairments in the domain of social awareness, which were more affected in older children with SCT. This developmental effect could possibly account to a certain degree for the relative strength of social awareness and motivation we detected in young children with SCT. It is therefore important that future studies examining the impact of SCT on the social functioning and related psychopathology, use a developmental approach, given the detailed information it provides on the impact of SCT at different developmental stages of life. This knowledge can be used in developing specific age-related preventive intervention strategies for young children with SCT aimed to support social development.

Relative to SCT groups bearing an extra X chromosome, we found a particularly pronounced vulnerability for social impairments in boys with XYY with regard to social cognition, social communication and restricted interests and repetitive behavior, but not for social awareness and social motivation. These findings suggest, on average, a more evident profile of social impairments in XYY associated with a typical ASD behavioral profile, and are consistent with earlier studies that compared social impairments across SCT karyotypes. For example, it was found that boys with XYY have a higher risk for clinical diagnoses of ASD, compared to boys and girls with an extra X chromosome (Cordeiro et al., 2012; Ross et al., 2012; Tartaglia et al., 2017; Wilson et al., 2019).

Several neurocognitive mechanisms may underlie the difficulties in social interaction

behavior under different conditions of social load, and risk on social impairments as observed in the current study. In the current study, we explored the role of global cognitive and language abilities in early social behavior (observed social interactions and daily life social impairments). First, for observed social interactions, we found that global cognitive level and receptive language skills were not associated with social interaction profiles. Although expressive language skills were correlated with level of social interactions, the ability of children to express themselves through verbal communication could not explain the difference between young children with SCT and their typically developing peers when it comes to social interaction behavior. Second, with regard to social impairments in daily life, these impairments showed to be correlated with global cognitive level and expressive language skills, but not with receptive language abilities. Also for social impairments, cognitive level and expressive language abilities could not explain the difference between the SCT and control group. Our results reveal that social impairments were found in both low IQ and average IQ groups, although particularly pronounced in children with SCT with a below average IQ. We can conclude that SCT has a specific impact on early social development, independent of the level of global understanding of the context and the ability to communicate with others in using language.

Our findings should be considered in light of several limitations. First, the majority of individuals with SCT still remains undetected during life (Berglund et al., 2019), although the group of diagnosed young children is rapidly growing with the introduction of the NIPT. In this study, social outcomes were not dependent on recruitment strategy (i.e. prospective follow-up group, information seeking parents group, or clinically referred cases group), which suggests that our findings are representative for this group of diagnosed children. However, it remains unsure to what degree the findings in this study can be generalized to those who have SCT, but remain undiagnosed. Second, although a considerable percentage of boys with XYY (Klinefelter Syndrome) received testosterone treatment (48%), we were unable to investigate the effect of testosterone treatment on social outcomes in the XXY group. Studies with suitable designs (randomized and placebo-controlled trials) could give insights into the effects of testosterone on behavioral outcomes in young boys with Klinefelter Syndrome.

Despite the above limitations, the current study with a relatively large and

international sample of young children with SCT provides a more detailed understanding of the early impact of SCT on social adaptive development. Our findings reveal that difficulties with social interactions arise already early in life of children with SCT. We found that level of social load is associated with specific types of deficits, in terms of lower social interaction attempts and more withdrawn behavior that were most pronounced when the level of social load was high. These observed social vulnerabilities during structured interactions showed to extend to daily life, as we found increased levels of social impairments that are associated with ASD. These findings suggest that the extra X and Y chromosome impact social adaptive development from a very early age, and stresses the importance of early routine monitoring and (preventive) support of social development and risk on social impairments related to ASD in young children with SCT.

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CHAPTER 4

The impact of Sex Chromosome Trisomies (XXX, XXY, XYY) on early social cognition: Social Orienting, Joint Attention and Theory of Mind

Bouw, N., Swaab, H., Tartaglia, N., & Van Rijn, S. (2022). 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, 37*(1), 63-77.

Abstract

Objective: About 1:650-1000 children are born with an extra X or Y chromosome (XXX; XXY; XYY), which results in a Sex Chromosome Trisomy (SCT). This study aims to cross-sectionally investigate the impact of SCT on early social cognitive skills. Basic orienting towards social cues, joint attention and Theory of Mind in young children with SCT were evaluated.

Methods: 105 children with SCT (range 1-7 years old) were included in this study, as well as 96 age-matched non-clinical controls. Eyetracking paradigms were used to investigate eye gaze patterns indicative of joint attention skills and orienting to social interactions. ToM abilities were measured using the subtest Theory of Mind of the NEPSY-II neuropsychological test battery. Recruitment and assessment took place in the Netherlands and in the United States.

Results: Eyetracking results revealed difficulties in children with SCT in social orienting. These difficulties were more pronounced in children aged three years and older, and in boys with 47,XYY. Difficulties in joint attention were found over all age groups and karyotypes. Children with SCT showed impairments in ToM (26.3% in the (well) below expected level), increasing with age. These impairments did not differ between karyotypes.

Conclusions: An impact of SCT on social cognitive abilities was found already at an early age, indicating the need for early monitoring and support of early social cognition. Future research should explore the longitudinal trajectories of social development, in order to evaluate predictive relationships between social cognition and outcome later in life, in terms of social functioning and the risk for psychopathology.

Introduction

Sex Chromosome Trisomy (SCT) is a common genetic variation, characterized by an extra X or Y chromosome, which results in the chromosomal patterns 47,XXY (Klinefelter Syndrome; KS), 47,XXX (Trisomy X or Triple X) or 47,XYY (XYY Syndrome), different from the typical 46,XY or 46,XX karyotype in boys and girls. Prevalence estimates vary from 1:650 to 1:1000 (Boyd et al., 2011; Berglund et al., 2019). Although the social behavioral phenotype is variable, associated social functioning might include difficulties with social interactions and social adjustment, social anxiety and shyness. Children and adolescents with SCT also show higher percentages of psychosocial challenges including difficulties in adaptive social functioning (Urbanus et al., 2020; Van Rijn et al., 2014; Van Rijn, 2019; Visootsak et al., 2009). On average across studies, 18% of children with XXY, 15% of girls with XXX and 30% of boys with XYY meet the criteria of a clinical diagnosis of Autism Spectrum Disorder (ASD; see for a review Van Rijn, 2019).

The presence of an extra X or Y chromosome is known to impact maturation of the brain structures within the networks that are referred to as the 'social brain' (Raznahan et al., 2016). Brain areas that are affected by the presence of an extra X or Y chromosome include the medial prefrontal, anterior cingulate and superior temporal sulcus, which play a key role in complex social cognitive functions such as Theory of Mind abilities (Soto-Icaza et al., 2015). Theory of Mind (ToM; also called 'perspective taking' or 'cognitive empathy') is a multifaceted construct defined as 'the ability to make the implicit assumption that the behavior of others is determined by their desires, attitudes and beliefs' (Frith & Frith, 2003). It is a core social cognitive ability, crucial in everyday life, needed to fully understand social interactions and a prerequisite for showing social adaptive behavior, responsive of social feedback following interactions. To understand the vulnerabilities of individuals with SCT in the development of adaptive social functioning, it is important to investigate the early impact of SCT on major aspects of social cognition in early childhood (Happé & Frith, 2014): social orienting, joint attention and ToM.

These early social cognitive functions have differential developmental trajectories and unfold at different stages of development, depending on brain maturation (Soto-Icaza, Aboitiz & Billeke, 2015). Typically from the age of three onwards the understanding of the complexity of social interactions that lead to another person's

false beliefs develop (Wellman et al., 2001; Devine et al., 2014). This increasing comprehension of false beliefs is represented in the levels of ToM, such as the level that refers to the understanding of second-order beliefs and the recognition of influence of earlier experiences on mental states (Wellman, 2014).

Fundamental in development of ToM are more basic social cognitive skills, such as joint attention (Sodian & Kristen-Antonow, 2015). Joint attention is defined as 'the capacity to coordinate attention between interactive sociwal partners with respect to objects or events in order to share an awareness of these objects or events' (Nation & Penny, 2008). Joint attention is associated with the early emergence of children's awareness that others have intentions, and is crucial for developing perspective-taking skills (Mundy & Newell, 2007). Experiencing the mental state of attention in episodes of shared attention is essential to the understanding of others' mental states. Joint attention is therefore considered essential in the development towards ToM (Korhonen et al., 2014). It includes the cognitive abilities of sharing attention (i.e. alternating eye-gaze), following the attention of another (i.e. following eye gaze or pointing) and directing the attention of another (Dawson et al., 2004). In the current study we focus on the impact of SCT on the ability to follow eye gaze or pointing of a social partner.

Even before children acquire more complex social cognitive functions like ToM, social cognitive development begins to be expressed in infancy with basic social perceptual abilities such as the ability to orient towards important social cues (i.e. faces, eyes). This ability is defined as social orienting (Mundy & Neal, 2001). This inborn elemental alignment of sensory receptors to a person or social event is believed to be the initial stage of the later developing more complex ability to think and reason about own or another person's intentions or mental state. Impairments in social orienting already early in life can deprive a child of social information input, which in turn could disrupt brain development and social cognitive development (Mundy & Neal, 2001).

There have been no studies investigating ToM, joint attention and social orienting in young children with SCT younger than eight years old. Only a few studies investigated ToM abilities in school-aged children and adolescents with SCT. These studies showed that ToM in school-aged boys and girls with 47,XXY and 47,XXX

was less well developed compared to typically developing controls (Van Rijn et al., 2014; Melogno et al., 2019). With regard to social orienting, it was reported that some individuals with SCT have deficits in attending to social cues: research in adult men with 47,XXY and in boys and girls with an extra X chromosome (47,XXX and 47,XXY) showed reduced attention to essential facial features and a missing typical first fixation on the eyes, both during the scanning of static facial expressions (Van Rijn, 2015), and during dynamic presentation of faces in emotional movie clips (Van Rijn et al., 2014). It is important to evaluate whether the impact of SCT on these social cognitive functions can already be detected early in development when these abilities typically develop (Soto-Icaza, Aboitiz & Billeke, 2015). Insight in the early impact of SCT on core social cognitive abilities early in life may help to understand the social difficulties that may emerge in later childhood, adolescence and adulthood of individuals with SCT. Second, knowledge about the impact of SCT on early social cognitive abilities might eventually be useful in identifying targets for early monitoring and (preventive) support of young children of SCT.

The aim of the current study was to investigate the early impact of SCT on social orienting, joint attention and ToM. Cross-sectional age differences were explored to understand developmental tracks. Objective and sensitive eyetracking measures were used to study social orienting and joint attention, as eye fixations can indicate how social information is processed. Based on the relevance of the X/Y chromosomes for development of neural networks supportive of the development of social cognition, we hypothesized that young children with SCT would show higher rates of difficulties with ToM, joint attention skills and social orienting, compared to their typically developing peers.

Materials and methods

Participants

The present study is part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study – Leiden, The Netherlands), which includes children with SCT and nonclinical controls aged 1–7,5 years. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome.

A group of 105 children with SCT (range 1-7.5 years old; M_{age}= 3.69, *SD* = 1.95) was

included in this cross-sectional study, as well as a population-based sample of 96 non-clinical controls within the same age range (42 boys; M_{age}= 3.62, *SD* = 1.63). Mean age did not differ between groups (t (199) = 0.26, p = .792). The SCT group consisted of 34 girls with 47,XXX (32.4%), 49 boys with 47,XXY (46.7%) and 22 boys with 47,XYY (21.0%). Seventy children (66.7%) were diagnosed prenatally (20 girls with XXX, 35 boys with XXY, 15 boys with XYY), and 35 children (33.3%); postnatally (14 girls with XXX, 13 boys with XXY, 8 boys with XYY). Twenty-four out of 49 boys with 47,XXY had received testosterone treatment (49.0%). The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping.

Recruitment and assessment took place on two sites: the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, The Netherlands, and the eXtraordinary Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado in Aurora, USA. Children in the SCT group were recruited in cooperation with the clinical genetics departments (from The Netherlands and Colorado (USA)), as well as through patient-advocacy groups and social media postings. For the SCT group, possible recruitment bias was assessed and three subgroups were identified: (1) 'active prospective follow-up', which included families who were actively followed after prenatal diagnosis (51.4% 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 (29.5% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development (19.0% of the SCT group). All participants had Dutch or English as first speaking language, had normal or corrected-to-normal vision, and did not have a history of traumatic brain injury. Non-clinical control children were recruited from the western part of The Netherlands, and approached with information brochures about the study. 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 ~1 in 1000, the risk of having one or more children with SCT in the control group was considered minimal and acceptable.

Age of the primary caregiver and parental education were assessed. Age of the primary caregiver ranged from 23 to 50 years. There was a significant difference

between research groups for age of primary caregiver (p <.001): on average, the primary caregiver of children with SCT was older (M = 38.80, SD = 4.75), compared to the primary caregiver of typically developing peers (M = 35.14, SD = 5.25). 96% of all parents indicated that their child has a second caregiver. Parental education was assessed according to the criteria of Hollingshead (Hollingshead, 1975). Scores of this scale include: 0 (no formal education), 1 (less than seventh grade), 2 (junior high school), 3 (partial high school), 4 (high school graduate), 5 (partial college or specialized training), 6 (standard college/university graduation), and 7 (graduate/professional training). If two parents were available, level of education was averaged over both parents. A Pearson χ^2 test was performed to investigate possible differences in parental education distribution between the SCT and control group. A significant difference was found ($\chi^2(10) = 18.43$, $p = .048$), indicating higher parental education in the SCT group. Average parental education was 5.90 (SD = 0.94) in the SCT group and 5.49 (SD = 1.36) in the control group.

Global level of cognitive development

To measure global level of intelligence and receptive language development, age appropriate instruments were administrated. The Bayley, 3rd edition (subscale cognitive scale; Bayley-III, 2009) was administered to 1-2 year old children. In the older children four subtests of the Wechsler Preschool and Primary Scales of Intelligence, 3rd edition (WPPSI-III; Wechsler, 2002) were used to estimate global level of intelligence (children aged 3 years: Block Design, Receptive Vocabulary, Information, Object Assembly; children aged 4 years and older: Block Design, Matrix Reasoning, Vocabulary, and Similarities). For children aged 4 years and older, Total IQ estimates were calculated based on this short form version of the WPPSI-III (Hurks et al., 2016). The Peabody Picture Vocabulary Test, 3rd edition (PPVT-III; Dunn & Dunn, 1997) was used to measure receptive language level in children aged 3 years and older.

Theory of Mind

The ToM subtest of the Developmental NEuroPSYchological Assessment, second edition (NEPSY-II; Korkman et al., 2007) was used to assess children's understanding of mental functions and other people's perspectives. The ToM subtest was administered to all participants aged three years and older. Also in this subset

of participants, mean age did not differ between the SCT and control group (t (126) = 1.70, p = .092).

The ToM subtest of the NEPSY was administrated according to the detailed instructions as described in the manual (Korkman et al., 2007). The ToM subtest consists of two different subtasks: verbal tasks and contextual tasks. In the verbal tasks, the questions are based on verbal scenarios. They measure the understanding of (false) beliefs, intentions, other's thoughts, ideas and comprehension of figurative language. Two items aim to measure the child's verbal and gestural imitation abilities, as imitation abilities are thought to be a basic ability for ToM skills. The child is asked to answer the tasks verbally, with the exception of an imitation question where the child is asked to imitate gestures or words. In all of the items the child can answer very briefly; one word is often sufficient for a correct answer, and in two of the questions it is also possible to answer by pointing. The contextual tasks of the ToM subtest aim to measure the child's ability to relate affects to a broader social context. In these items the child is shown drawings with children in social contexts. In each drawing there is a target girl whose face is not shown. The child is asked to point to one of four photographs of the same girl's face with different emotions selecting the emotion of the girl in the drawing.

The total score range is between 1 and 28 (sum score of the 15 verbal tasks and 6 contextual tasks), with higher scores reflecting better ToM skills. Besides raw scores, percentile scores as compared to norms from the general population can be calculated; depending on the spoken language of the child, the Dutch or English norms were used. Percentile scores were labeled as being in the normal range (percentile score > 25), the borderline range (11 < percentile score ≥ 26), the below expected level (3 < percentile score > 10), and the well below expected level (percentile score ≤ 2).

Joint Attention

Joint attention was assessed during a paradigm similar to Von Hofsten et al. (2005) and Falck-Ytter et al. (2012). The children were shown video clips of a female adult sitting behind a table. On the table four distinct objects were presented: a bowling set, a train, a camera, and a ball. Since gazing and pointing are both important attention-directing elements of joint attention, these elements are both part of

the paradigm, in which they were presented separately as well as combined: the adult just looked at the object (gaze; G condition) or just pointed while looking straight ahead (point; P condition), or both looked and pointed (gaze and point; G+P condition). The adult performed the action for four seconds, after which she returned her attention to the camera. The clips lasted 8.5-10 seconds, did not include speech, and background music was added to keep the children involved in the task. To prevent carry-over effects, a black slide was presented for two seconds between clips. The order of clips was counterbalanced between participants. See Figure 1 for screenshots of a trial in the G+P condition. As a measure of the tendency to accurately follow referential cues, a difference score (DS) was calculated. DS represents the standard measurement of the ability of children to follow gaze (Moore & Corkum, 1998), and reflects the number of gaze shifts from the adult to the specific area of interest (AOI) of the attended object minus the number of gaze shifts from the adult to the AOIs of the unattended objects (Gredebäck et al., 2010).

Figure 1. Screenshots of the video clips in the Joint Attention Paradigm (1), and the Social Orienting Paradigm (2).

Social Orienting

Eye gaze fixations towards key sources of social information, namely faces and eyes, were measured during a Social Orienting Paradigm. See Figure 1 for a screenshot of the video clip. The 30 second video showed a social plot, in which social cues are reciprocally exchanged between two persons. A child and adult are seated on chairs with a table in between, with non-social distractors (toys) in the background. The social 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. Then, the adult places the chocolate in one of her closed hands,

shows her closed hand, and asks the child to guess in which hand she is holding the chocolate; the child correctly identifies the hand with the chocolate, and the adult shows the chocolate. However, unexpectedly the adult does not allow the child to take the chocolate. The child shows sadness and disappointment.

In order to preserve ecological validity, all sounds were retained. To prevent interference with language abilities, language used in the clip was not the same as the language of the participants (i.e. Italian vs. Dutch/English). In a group of non-clinical young children aged 3-7 years, this eyetracking paradigm was found to be related to real-life social behaviors, and independent of age, IQ, or gender (Van Rijn et al., 2019).

Eyetracking equipment and procedures

Gaze data within specific AOIs were collected using the Tobii X2-60 eyetracker (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 computer with eyetracker was placed on a table adapted to the height of the seat, and the child was seated in a car seat at 65 cm viewing distance. A 5-point calibration procedure was used, with successful calibration defined as a maximum calibration error of 1 degree for individual calibration points (i.e. < 1 cm at a distance of 65 cm from the eyetracker). After the calibration procedure, the child was instructed to watch the movie clips and pictures on the computer. The two eyetracking paradigms started with an attention grabber (e.g. a moving picture of an animal, shown on a black background and accompanied by a sound) to direct the attention of the child to the screen.

Gaze data was processed using Tobii Studio (version 3.2.1), using the Tobii Identification by Velocity Threshold (I-VT) fixation filter. This filter controls for validity of the raw eyetracking data making sure only valid data were used (Olsen, 2012).

The 'Dynamic AOI' tool of Tobii Studio was used to draw AOIs, which were drawn with a one centimeter margin to ensure that the AOIs were sufficiently large outside the defining contours to reliably capture the gaze fixation (Hessels et al., 2016). Dynamic AOIs were grouped into the following categories: adult, attended and unattended

objects, and the whole screen (Joint Attention paradigm); faces, eyes, and the whole screen (Social Orienting Paradigm). Eyes were included in the face AOI to prevent overlap, in terms of reliably distinguishing the impact of SCT on orienting to faces and eyes. In order to evaluate the amount of nonvalid eye tracking data (i.e. attention toward the screen), the total visit duration toward the whole screen was calculated, divided by the duration of the clip, multiplied by 100, reflecting the percentage of valid data collected during each of the eye tracking paradigms.

In the Joint Attention Paradigm, the amount of gaze shifts was based on the individual gaze plots of the participants (provided by Tobii Studio), combined with the amount of fixations on the AOIs (*N* Fixation count). We included gaze shifts from fixations on the AOI adult to fixations on any of the four possible reference AOIs, occurring after the time the adult's gaze or pointing gesture was first directed at the attended object. In the Social Orienting paradigm, proportions fixation duration were calculated by taking the total fixation duration within the AOI, divided by the total visit duration toward the whole screen of the individual child, multiplied by 100, reflecting the percentage of time children were attending to an AOI.

Study procedures

Signed informed consent was obtained from the parents of all participating children, according to the declaration of Helsinki. 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. Assessment took place at various sites (Colorado, USA and The Netherlands) 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 at both sites. Researchers from Leiden University were responsible for project and data-management (i.e., training and supervision of researchers processing and scoring of data). Administration of the NEPSY, WPPSI was performed on a table by trained child psychologists or psychometrists in the Dutch or English language, depending on the first language of the child. The eyetracking procedure and PPVT administration took place during a separate appointment, within one week after the NEPSY and WPPSI administration. The laptop with the eyetracker was placed in a small tent to standardize the testing environment, and to control for light conditions. The

child was seated in a car seat in front of the eyetracker. The examiner was seated beside the child (directing Tobii Studio with a remote keyboard), and started the calibration procedure. Eyetracking paradigms were shown in a fixed order (social orienting/joint attention). Parents were allowed to stay in the room (out of sight) and were asked not to communicate with their child during the procedure.

Data analyses

Statistical Package for the Social Science (SPSS) version 25 was used for statistical analyses. Pearson's correlations were used in order to test for associations between social orienting, joint attention, ToM, cognitive functioning and receptive language abilities. For group wise (SCT vs. control; research site; recruitment bias) comparisons of ToM skills, proportions of fixation duration within the AOIs, independent *t*-tests or MANOVAs were used. 3x2 ANOVA were used to assess the main effect of condition (Gaze, Point, Gaze+Point) on joint attention outcomes in the SCT vs. control group. The moderating effect of age on the differences of social orienting, joint attention and ToM between the SCT and control group, was assessed using a bootstrapping, nonparametric resampling procedure (PROCESS; Hayes, 2009). Bootstrapping analysis with 5000 resamples was done to test for a significant moderating effect using the SPSS macro developed by Hayes (2017). In this analysis, the moderation effect is significant if the 95% bias corrected confidence interval for the moderator effect does not include zero. A Pearson χ^2 test was used to explore the distributions of karyotypes across age groups that were created in order to measure SCT vs. control differences in ToM and social orienting. Influence of karyotype on social orienting, joint attention and ToM was tested by an MANOVA. Statistical analyses were performed one-tailed (SCT vs. control) or two-tailed (influence of research site, karyotype, recruitment bias), and level of significance was set at $p < .05$. In case of significant differences, Cohen's *d* or partial η^2 were used to calculate effect sizes.

Results

Differences in early social cognition between research sites

First, to control for the potential impact of research site on outcomes of the study, the data of the two research sites were compared. No differences between children in The Netherlands and USA were found for ToM (t (61) = 1.32, p = .192), joint attention

(t (89) = 0.80, p = .428), and social orienting (faces: (t (89) = 0.234, p = .816); eyes: (t (89) = 0.68, p = .499). Based on this, all SCT data were collapsed across sites.

Theory of Mind

ToM: differences in SCT vs. controls. The NEPSY ToM task was completed by 128 children (9 children were not able to complete the task due to fatigue of the child). Independent t-tests indicated differences between the SCT and control group for verbal ToM (t (126) = -1.75, p = .041. Cohen's d = .31), contextual ToM (t (126) = -2.42, p = .009. Cohen's d = .43), and total ToM (t (126) = -2.42, p = .013. Cohen's d = .40), indicating lower ToM scores in the SCT group. The effect size (Cohen's d) on the total score differences between groups indicated medium SCT vs. control differences. See Table 1 for the exact mean and SD values for the Verbal ToM scores, the Contextual ToM scores and total scores. When evaluating scores normalized for age, for overall ToM skills, 47.5% of the SCT group scored in the average to above average level, 26.2% scored in the borderline level, 19.7% in the below expected level, and 6.6% in the well below expected level.

Table 1. Descriptive statistics for SCT and control group.

	Age group	*N*	Missing	SCT		Control	
				Min-Max	*M (SD)*	Min-Max	*M (SD)*
Cognitive development (norm score; Bayley-III)	1-2 year	63	1	62-125	98.52 (13.24)	70-129	99.43 (14.98)
Total IQ (WPPSI-III)	3-7 years	131	6	55-138	95.17 (19.57)	72-140	109.15 (13.56)
Receptive language (standard score; PPVT-III)	3-7 years	132	5	65-129	99.46 (14.75)	74-133	108.67 (12.67)
Social Orienting to faces (proportions fixation duration)	1-7 years	186	15	.00 - .64	.22 (.12)	.00 - .57	.24 (.12)
Social Orienting to eyes (proportions fixation duration)	1-7 years	186	15	.00 - .38	.05 (.06)	.00 - .28	.08 (.07)
Joint Attention (difference score gazeshifts)	1-7 years	187	14	-6.33- 4.33	-0.56 (2.29)	-3.67-7.67	1.31 (2.27)
Theory of Mind Verbal (raw score; NEPSY-II)	3-7 years	128	9	0-16	7.22 (4.03)	1-21	8.60 (4.82)
Theory of Mind Contextual (raw score; NEPSY-II)	3-7 years	128	9	0-6	2.41 (1.36)	0-6	3.06 (1.65)
Theory of Mind Total Score (raw score; NEPSY-II)	3-7 years	128	9	0-19	9.49 (4.71)	3-24	11.66 (6.01)

Note: SCT = Sex Chromosome Trisomy.

ToM: age dependent differences in SCT versus controls. A bias-corrected bootstrapping analyses (PROCESS) were conducted to test for a moderating effect of child's age on the difference in total ToM skills between the SCT and control group. There was a significant moderation effect of child's age (b = 2.69, *SE* = 0.57, *t* = 4.67, *p* <.001, 95% CI [1.55, 3.83]), revealing that the difference on total ToM skills between the SCT and control group increases with older ages (not in favor of children with SCT). R^2 = .59, indicating that this model explained 59% of the variance in ToM skills. See Figure 2 for a graphical representation of ToM in the SCT vs. control group.

Mean raw scores were significantly correlated with general IQ (measured with WPPSI Total IQ; Pearson's *r* = .25, *p* = .006), and normed scores on a receptive language skills task (measured with the PPVT; *r* = .62, *p* < .001). In order to control for the association between ToM task performance and cognitive or receptive language skills, norm scores of global intelligence and receptive language skills were added as covariates to the PROCESS analysis. The significant effect of group on ToM skills remained, even when global intelligence and receptive language skills were added as covariates. A significant moderation effect of child's age was found (b = 1.32, *SE* = 0.60, *p* = .030, 95% CI [0.13, 2.52], R^2 = 0.67), revealing that the difference on ToM skills between the SCT and control group increases with older ages, while controlling for global intelligence and receptive language skills.

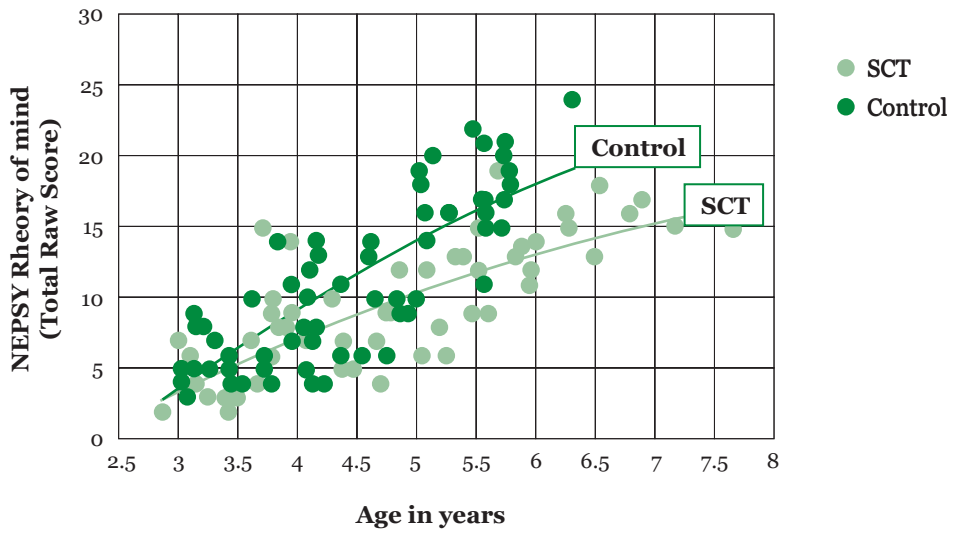

Figure 2. Theory of Mind and age for the Sex Chromosome Trisomy and control group.

ToM: differences in SCT vs. controls in two age groups. Because of these age and group effects, participants were divided in two age groups in order to investigate ToM abilities in different stages during development in early childhood: children 3 and 4 years old (n = 75; M_{age} = 3.91, SD_{age} = 0.59; 34 SCT (10 XXX, 19 XXY, 5 XYY), 41 controls), and children 5, 6 and 7 years old (n = 53; M_{age} = 5.87, SD_{age} = 0.68; 29 SCT (15 XXX, 9 XXY, 5 XYY), 24 controls). There was no significant association between the type of SCT and age group (χ^2 (2) = 4.20, p = .122), indicating that the distribution of karyotypes was similar across age groups.

There was no difference in total ToM score in children aged 3-4 years in the SCT group (M= 6.85, SD=3.24) and the control group (M=8.05, SD= 3.80; t (73) = -1.45, p = .076). In the 5-7 years old age group a difference was found between the SCT (M=12.59, SD= 4.29) and control group (M=17.83, SD = 3.54; t (51) = -4.79, p <.001; Cohen's d = 1.33): 5-7 years old with SCT showed lower ToM abilities compared to their typically developing peers.

Joint Attention

Joint attention: attention toward the screen. The Joint Attention Paradigm was successfully completed by 190 children (11 children were not able to complete the task, due to technical issues or fatigue of the child). The total proportion of valid on-screen visit duration was 70.4% for the whole eye tracking paradigm, and did not differ between the SCT and control group (t (188) = -1.25, p = .215). This proportion indicated sufficiently high attention to the screen (> 70%; Frank et al., 2014). The main outcome measures, i.e. Difference Scores (DS) for the three conditions (Gaze, Point, Gaze+Point) were not correlated with intellectual functioning (p =.081; Bayley cognition scaled score or WPPSI Total IQ).

Joint attention: effect of condition. A 3x2 mixed ANOVA with DS as the dependent variable with attention-direction condition (Gaze/Point/Gaze+Point) as within factor and group (SCT, control) as between subject factor revealed no significant main effect of condition (F (2,184) = 1.70, p = .186), and no significant interaction effect (F (2,184) = 1.62, p = .200), indicating no effect of condition on gaze following. Therefore, the three conditions were collapsed in subsequent analyses.

Joint attention: differences in SCT vs. controls. An independent *t*-test with DS as dependent variable showed a significant effect of research group (SCT vs. control; $t(185) = -5.60$, $p < .001$, Cohen's $d = 0.82$). These effects reflect lower joint attention accuracy in the SCT group over all ages ($M = -0.56$, $SD = 2.29$), compared to the control group ($M = 1.31$, $SD = 2.27$).

Joint attention: age dependent differences in SCT vs. controls. A bias-corrected bootstrapping PROCESS analysis was conducted to test for a moderating effect of child's age on the difference in DS between the SCT and control group. There was no significant moderation effect of child's age (b = 0.15, $SE = 0.16$, $t = 0.94$, $p = .349$, 95% CI [-0.17, 0.48]), revealing that the difference on DS between the SCT and control group was not moderated by age. See Figure 3 for a graphical representation for the SCT vs. control group.

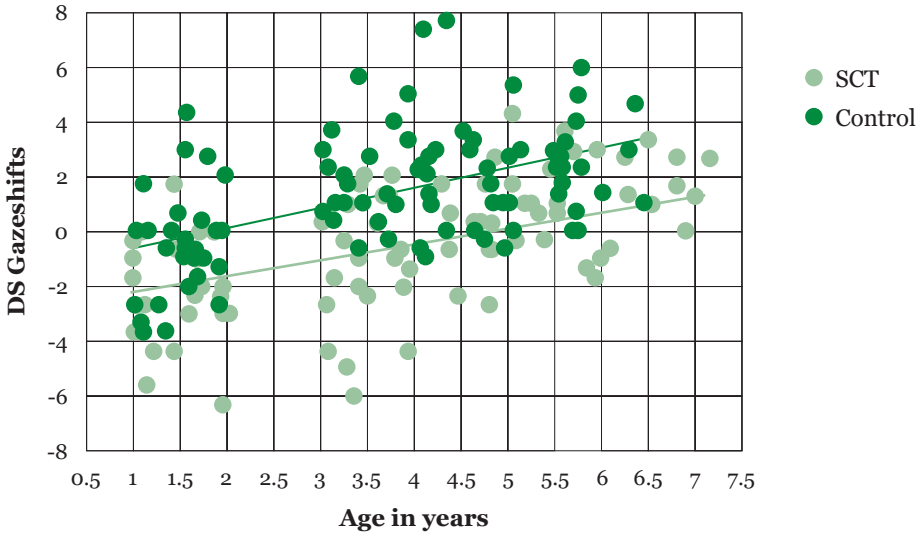

Figure 3. Joint attention: DS Gazeshifts and age for the Sex Chromosome Trisomy and control group.
Note: DS Gazeshifts: difference scores between gaze shifts from AOI Adult to AOI Attended object minus gaze shifts from AOI Adult to AOI Unattended object.

Social orienting

Social orienting: attention towards the screen. The Social Orienting Paradigm was successfully completed by 186 children (15 children were not able to complete the

task due to technical issues or fatigue of the child). The total proportion valid on-screen visit duration was 94.1%, and did not significantly differ between the SCT and control group (t (184) = -1.77, p = .079). The main outcome measures were not correlated with cognitive functioning (proportions of fixation for the AOI faces (p =.495) and eyes (p =.155; dependent on age: Bayley cognition scaled score or WPPSI Total IQ).

Social orienting: differences in SCT vs. controls. Total eye gaze fixation durations at AOI faces and eyes were measured in order to investigate SCT vs. control differences in social orienting. Independent t-tests on eye gaze fixation duration at AOI faces and eyes indicated that on average, children with SCT showed lower fixation duration to eyes (t (184) = -3.01, p = .002, Cohen's d= .46), but not to faces (t (184) = -1.33, p = .093). See Table 1 for exact M and SDs.

Social orienting: age dependent differences in SCT versus controls. A bias-corrected PROCESS bootstrapping analysis was conducted to test for a moderating effect of child's age on fixation duration on the AOI Faces and Eyes between the SCT and control group. There was a significant moderation effect of child's age on fixation durations on the AOI Faces (b = 0.02, SE = 0.01, t = 2.32, p = .022, 95% CI [0.004, 0.044]; R^2 = .05), but not on the AOI Eyes (b = 0.009, SE = 0.006, t = 1.68, p = .094, 95% CI [-0.002, 0,020]).

Social orienting: differences in SCT vs. controls in three age groups. Because of these group and age effects, the participants were divided in three age groups in order to further investigate eyetracking outcome measures in different stages during development in early childhood: children 1 and 2 years old (n = 59; M_{age} = 1.48, SD_{age} = 0.33; 29 SCT (6 XXX, 19 XXY, 4 XYY), 30 controls), children 3 and 4 years old (n = 74; M_{age} = 3.91, SD_{age} = 0.60; 34 SCT (11 XXX, 17 XXY, 6 XYY), 40 controls), and children 5, 6 and 7 years old (n = 53; M_{age} = 5.84, SD_{age} = 0.64; 28 SCT (15 XXX, 8 XXY, 5 XYY), 25 controls). There was no significant association between the type of SCT and age group (χ^2 (4) = 8.22, p = .084), indicating that the distribution of karyotypes was similar across age groups.

There were no overall SCT vs. controls differences in fixation duration at the faces and eyes AOI in children aged 1-2 year (V = 0.061, F (2,56) = 1.81, p = .087), and the

3-4 years age group (F (2,71) = 1.57, p = .108). In the 5-7 years old age group a SCT vs. control difference was found (F (2,50) = 7.313, p = .001; partial η^2 = 0.23): 5-7 years old with SCT fixated less at faces and eyes than the control group. See Table 2 for exact M and SD, p-values, and post-hoc effects.

Table 2. Groups differences in social orienting (SCT vs. control) in fixation duration in three age groups (M, SD).

	Phases of development												
	1, 2 years old			3, 4 years old					5, 6, 7 years old				
	n = 59 (29 SCT, 30 control)			*n* = 74 (34 SCT, 40 control)					*n* = 53 (28 SCT, 25 control)				
AOI	SCT	Control	*p*-value	SCT	Control	*p*-value	Post-hoc effect	Cohen's *d*	SCT	Control	*p*-value	Post-hoc effect	Cohen's *d*
Faces	.26 (.15)	.23 (.16)	.189	.20 (.11)	.23 (.10)	.156			.19 (.10)	.28 (.09)	<. 001	SCT < control	.95
Eyes	.05 (.08)	.06 (.07)	.283	.05 (.05)	.07 (.07)	.040	SCT < control	.33	.06 (.06)	.12 (.07)	<. 001	SCT < control	.92

Note : SCT = Sex Chromosome Trisomy; AOI = Area of Interest.

Associations between social cognitive skills within the SCT group

To explore associations between basic social cognitive skills and ToM within the SCT group, Pearson's correlations were calculated between social orienting, joint attention and ToM. A significant correlation was found between social orientation to faces and joint attention (r = .217, p = .045), and a correlation for joint attention and ToM (r = .331, p = .010). See Table 3 for r-values for all variables.

Table 3. Intercorrelations for social orienting, joint attention and Theory of Mind in the SCT group.

	1.	2.	3.	4.
1. Social orienting to faces	-	.626**	.217*	.943
2. Social orienting to eyes		-	.124	.124
3. Joint attention			-	.331**
4. Theory of Mind				-

Note: SCT = Sex Chromosome Trisomy;* p <.05; ** p <.01.

Karyotype differences within the SCT group

In order to investigate the influence of various karyotypes on social cognitive outcomes accounting for the effect of age, ANCOVA analyses were carried out with main effect of karyotype (XXX vs. XXY vs. XYY) and age as covariate. For ToM (N = 63; 25 XXX, 28 XXY, 10 XYY), no difference between karyotypes was found (F (2,59) = .47, p = .625). Also for Joint Attention skills (N = 91; 30 XXX, 46 XXY, 15 XYY), no difference between karyotypes was found (F (2,87) = .48, p = .623). For social orienting (N = 91; 32 XXX, 44 XXY, 15 XYY), no significant difference was found for orienting to eyes (F (2,87) = 2.06, p = .134). These results indicate that karyotype was not predictive of ToM and Joint Attention abilities, and social orienting to eyes, when accounting for age. However, a significant difference between karyotypes was found on social orienting to faces, (F (2,87) = 4.09, p = .020, partial η^2 = .09). Estimated Marginal Means revealed that the XYY subgroup had significantly decreased social orienting to faces compared to the XXY subgroup (p = .041), and XXX subgroup (p = .005).

Recruitment bias within the SCT group

Within the SCT group we tested for differences on social cognitive outcomes between the three recruitment groups with MANOVA. There were no significant differences for social cognitive outcomes (see Table 4): how children enrolled in the study did not affect their outcomes on ToM, joint attention and social orienting abilities.

Table 4. Differences in social cognition across recruitment groups (M, SD).

	Prospective follow-up	**Information seeking parents**	**Clinically referred cases**	*p*-value
NEPSY Theory of Mind	*n* = 29	*n* = 20	*n* = 14	
Total raw score	8.90 (4.97)	10.00 (4.46)	10.00 (4.71)	.658
Joint Attention paradigm	*n* = 46	*n* = 28	*n* = 17	
DS gaze shifts	16.33 (9.29)	20.32 (7.94)	19.29 (5.51)	.114
Social Orienting paradigm	*n* = 43	*n* =28	*n* =20	
Total fixation duration faces	.21 (.12)	.25 (.14)	.20 (.09)	.308
Total fixation duration eyes	.04 (.05)	.06 (.05)	.06 (.05)	.345

Note: DS = difference score.

Discussion

The present study was designed to evaluate the impact of SCT on early social cognitive development. As an extra X or Y chromosome impacts maturation of brain areas involved in social cognitive processes (Raznahan et al., 2016), and SCT increases the risks of difficulties in adaptive social functioning in childhood , adolescence and adult life (Van Rijn, 2019), we studied one of the core social cognitive skills, namely Theory of Mind, in addition to basic social cognitive skills typically developed during the first years of life, i.e. social orienting and joint attention. Insights in the impact of SCT on early social cognitive skills could enhance our understanding of how social behavioral difficulties may be anchored in altered processing of social information already early in neurodevelopment.

We addressed the question whether the impact of SCT on ToM abilities could already be found in children at 3 to 7 years of age. In line with our expectations, a difference

was found in overall ToM skills between children with SCT and their age related peers, suggesting that on average young children with SCT have difficulties with ToM. The proportion of young children with SCT that scored in the below expected level (19.7%) and well below expected level (6.6%) revealed that substantial ToM impairments already can be found in a subset of the SCT group early in development, across all SCT karyotypes. Next, when age was taken into account, analyses showed a moderating effect of age, indicating more ToM difficulties in older children with SCT. This effect remained significant even after controlling for global intelligence and receptive language level. From the age of 5 years old, children with SCT as a group showed impairments in ToM, with large effect sizes.

From a developmental perspective, it is known that basic social cognitive skills are involved in the maturation of ToM (Hughes & Leekam, 2004; Korhonen et al., 2014). We therefore studied two cognitive functions related to ToM with the help of eyetracking paradigms: 1) joint attention, i.e. being able to coordinate attention between a social partner with respect to objects or events, and 2) social orienting, i.e. the ability to align sensory receptors to social important cues. First, we found a difference with a large effect size in joint attention between the SCT and control group, suggesting difficulties with joint attention in children with SCT. These difficulties in joint attention were irrespective of age. Second, with regard to social orienting, eyetracking results showed that children from the age of 3 years on general are less inclined to orient their attention towards social cues, as compared to their typically developing peers. No social orienting differences between the SCT and control group were seen in children aged 1-2 year old.

The difficulties with ToM and contributing basic mechanisms of social orienting and joint attention as found in this study have implications for our understanding of social-adaptive problems found in young children with SCT (Urbanus et al., 2020), and social (cognitive) difficulties found later in life of children and adolescents with SCT (Urbanus et al., 2019). To be more specific, these findings may suggest that challenges in social functioning throughout the life span are likely anchored in social cognitive vulnerabilities that may be present already very early in life. At the age of 1-2, children with SCT on average showed intact social orienting, however, processing gaze as part of joint attention seems to be affected. Given that joint attention requires the adequate processing of eye gaze and gestures to follow

intentions of others, it is not only sufficient for young children to orient towards the social value of information, but also required to have awareness that others have intentions and perceptions (Charman et al., 2000).

At the age of three to five, on average, both social orienting and joint attention seem to be affected in young children with SCT. Failures to demonstrate the tendency to orient to social stimuli deprive children from access to social information needed to further develop skills in following, understanding, and responding to social directions and interactions of others. Studies with typically developing children have shown that basic social orienting skills and the ability to coordinate attention with a social partner in relation to a third object (i.e. joint attention) correlates with the development of the ability to decode and reason about others' mental state (i.e. ToM; Wellman et al, 2001; Sodian & Kristen-Antonow, 2015). Indeed, in our study we found impaired ToM abilities in children with SCT from the age of five, irrespective of their level of global intelligence and receptive language. As expected, social orienting, joint attention and ToM were shown to be associated: in the SCT group we found that social orienting to faces was significantly correlated with joint attention, and joint attention was correlated with ToM.

The phenomenon of increasing impairments of more complex abilities in older age groups of children with SCT in comparison with typically developing peers as found in this study with regard to social orienting and ToM is known as 'growing into deficit' (Rourke, 1983). Impairments in fundamental social cognitive abilities may lead to a cascade of negative developmental effects, as vulnerabilities with attending, following and understanding social information increase while the access to quantitative and qualitative opportunities to learn from social interactions decrease. This negative loop of increasing difficulties may impact the development of social adaptive behavior, involved in the forming and maintaining of reciprocal social relationships.

It is known that early impairments in social orienting, joint attention and ToM influence (social) development through childhood. In typically developing children the development of social cognitive skills seem to be associated with other domains of neurocognitive development, such as language (Delgado et al., 2002) and executive functioning (Drayton et al., 2011). These neurocognitive functions have also shown

to be vulnerable in individuals with SCT (see for a review: Van Rijn, 2019). To illustrate, the increasing ability of young children to use social information such as gaze direction and pointing to locate an object (i.e. joint attention) increases the opportunity for forming correct word-object associations, required for the acquisition of communicative competence and language (see Delgado et al., 2002). Studies have shown that difficulties with orienting towards social cues, joint attention and ToM early in development are also associated with Autism Spectrum Disorders (ASD; Baron-Cohen et al., 1992; Chawarska et al., 2003; Sullivan et al., 2007).

When exploring differences between karyotypes in SCT in impact on social orienting, joint attention and ToM at different ages, the results suggest that although social cognition was impaired in all karyotypes, social orienting difficulties might be somewhat more pronounced in boys with 47,XYY compared to boys and girls with 47,XXX and 47,XXY. On a behavioral level it has already been shown that boys with 47,XYY have a higher risk for ASD compared to boys with 47,XXY (Cordeiro et al., 2012; Ross et al., 2012; Tartaglia et al., 2017). This more pronounced vulnerability in the XYY group compared to the XXX and XXY group was not found in joint attention and ToM, which were similarly affected across all karyotypes.

Considering the importance of social orienting, joint attention and ToM for a broad range of developmental outcomes, further research will be needed to explore whether difficulties in ToM, joint attention and social orienting in young children with SCT are predictive of risk for later social adaptive difficulties and neurodevelopmental diagnoses such as ASD. The detection of early markers in young children with SCT (both in research and clinical practice) is crucial considering the large heterogeneity in children with SCT (see for example the heterogeneity in the behavioral profile of young children with SCT; Urbanus et al., 2020). Monitoring of children with SCT from infancy to childhood (through testing, relevant questionnaires completing by the surrounding, observations in natural settings), and if necessary preventive support should first focus on the basic ability to attend to social cues, and further on the ability to follow triadic communicative exchanges (i.e. joint attention), and complex ToM abilities from around the age of three. Currently available interventions available for children with ASD, aimed to increase the motivation to orient towards social stimuli, to be involved in triadic exchanges with a social partner, and to develop ToM abilities, might also benefit children with SCT. Future studies addressing the effectiveness of

(preventive) interventions in young children with SCT are warranted.

The current study has both strengths and limitations. Strengths included the relatively large, international sample of the study, that consisted of children varying in time of diagnosis (pre- or postnatal), recruitment strategy, and boys with 47,XXY that did or did not receive testosterone treatment. With the large sample size of this study, we were able to investigate social cognitive abilities of children at specific ages (i.e., 1-2, 3-5, and 5-7 age groups). Social cognitive outcomes in the SCT group did not differ across international research sites, indicating a high degree of similarity in social cognitive function among children with SCT in Western cultures. As it was beyond the scope of this study to investigate the influence of time of diagnosis (i.e. prenatal/postnatal), and testosterone treatment in boys with 47,XXY, future studies with suitable designs (e.g. Randomized Control Trials investigating the influence of testosterone) should study these parameters in relation with general social cognitive functioning in young children with SCT. An important limitation of the current study is the cross-sectional design, which limits cause-effect conclusions. Therefore, future research should further investigate the longitudinal effects of impairments in social orienting, joint attention and ToM on behavioral outcomes and psychopathology in young children with SCT, which will be explored in this population with prospective follow-up.

Further, these results demonstrate the performance of young children (both SCT and control) on tasks designed to measure early social cognitive skills which included stimuli presented on paper and screens. Although overall social functioning in naturalistic environments encompasses more than only neurocognitive behavior, the outcomes of the NEPSY task and eyetracking paradigms are found to be predictive for daily life social behavior (respectively: Rosello et al., 2020; Van Rijn, Urbanus, & Swaab, 2019).

In this study, social cognitive performance of young children with SCT was not dependent on recruitment strategy (i.e. prospective follow-up group, information seeking parents group, or clinically referred cases group), which suggests that our findings are representative for this group of diagnosed children. However, it is important to take into consideration that SCT is still highly underdiagnosed or diagnosed late in life (Berglund et al., 2019), although it is expected that more

individuals will be diagnosed early in life with the introduction of less invasive screening methods during pregnancy (Samango-Sprouse et al., 2017). Nonetheless, it remains unsure to what degree the findings in this study can be generalized to those who have SCT, but remain undiagnosed.

To conclude, the study presented here shows that already at an early age, SCT impacts the ability to orient to social information and to follow and understand the desires, believes and intentions of others. These difficulties are seen from an early age onwards and become increasingly deviant across the age range of three to seven. Knowledge about these early social cognitive abilities is important, as this may help to identify targets for early monitoring and preventive interventions.

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CHAPTER 5

The impact of Sex Chromosome Trisomies (XXX, XXY, XYY) on gaze towards faces and affect recognition: A cross-sectional eye tracking study

Bouw, N., Swaab, H., Tartaglia, N., Cordeiro, L., & Van Rijn, S. (2022). The impact of sex chromosome trisomies (XXX, XXY, XYY) on gaze towards faces and affect recognition: a cross-sectional eye tracking study. *Journal of Neurodevelopmental Disorders, 14*(1), 1-16.

Abstract

Objective: About 1:650-1000 children are born with an extra X or Y chromosome (47,XXX; 47,XXY; 47,XYY), which results in a Sex Chromosome Trisomy (SCT). This international cross-sectional study was designed to investigate gaze towards faces and affect recognition during early life of children with SCT, with the aim to find indicators for support and treatment.

Methods: A group of 101 children with SCT (aged 1-7 years old; Mage= 3.7 years) was included in this study, as well as a population-based sample of 98 children without SCT (Mage= 3.7). Eye gaze patterns to faces was measured using an eye tracking method that quantifies first fixations and fixation durations on eyes of static faces and fixation durations on eyes and faces in a dynamic paradigm (with two conditions: single face and multiple faces). Affect recognition was measured using the subtest Affect Recognition of the NEPSY-II neuropsychological test battery. Recruitment and assessment took place in the Netherlands and the United States.

Results: Eyetracking results reveal that children with SCT show lower proportions fixation duration on faces already from the age of three years, compared to children without SCT. Also, impairments in the clinical range for affect recognition were found (32.2% of the SCT group scored in the well below average range).

Conclusions: These results highlight the importance to further explore the development of social cognitive skills of children with SCT in a longitudinal design, the monitoring of affect recognition skills, and the implementation of (preventive) interventions aiming to support the development of attention to social important information and affect recognition.

Introduction

About 1:650 - 1000 children are born with an extra X or Y chromosome, which results in the chromosomal patterns 47,XXY (Klinefelter Syndrome; KS), 47,XXX (Trisomy X or Triple X) or 47,XYY (XYY Syndrome), as compared to the typical 46,XY or 46,XX karyotype in boys and girls. These sex chromosome trisomies (SCTs) are caused by a spontaneous nondisjunction of the X or Y chromosome during early cell division, and often not diagnosed (Boyd et al., 2011; Berglund et al., 2019). SCT is being increasingly identified during pregnancy as the consequence of recent technical advances of non-invasive prenatal screening (i.e. the introduction of the noninvasive prenatal screening test; NIPT). Therefore, the unique opportunity is present to gain insight in the developmental pathways and mechanisms that underlie developmental risks of very young children with SCT, an area of research that has not received much attention so far.

There is wide phenotypic variability among individuals with SCT, with an increased risk of somatic, neurodevelopmental, educational, behavioral, and psychological difficulties during development in childhood and adolescence and in adult life (Tartaglia et al., 2015). Neurocognitive challenges in childhood and adolescence include impairments in language development, social cognition, and executive functioning. Global intellectual functioning within SCT is variable, ranging from impaired to above average; mean intellectual functioning is in the average to low-average range (Urbanus et al., 2020). However, many studies only include adolescents and adults with SCT, and a majority focus on the somatic phenotype (Pieters et al., 2011). Social adaptive functioning of individuals with SCT has recently received more attention. Although the social phenotype is variable and varies widely within the SCT group, there is increasing recognition that individuals with SCT have an increased risk for social anxiety, difficulties with social interactions and social adjustment, and impairments in social cognitive abilities (Urbanus et al., 2020; Van Rijn et al., 2014; Van Rijn, 2019). Interestingly, neuroimaging studies in individuals with SCT have shown that the X and Y chromosomes impact brain networks involved in higher-order cognition (see for a review: Hong & Reiss, 2014). A neuroimaging study comparing the impact of the extra X and Y chromosome on cortical anatomy contribute to our understanding of neural mechanisms that underlie vulnerabilities of individuals with SCT on social cognitive domain, as it was shown that the presence of an extra X or Y chromosome convergently impacts the maturation of brain areas

within the 'social brain' network (Raznahan et al., 2016).

Insights in the development of social cognition help to understand vulnerabilities in social adaptive functioning (as described in the SOCIAL model: Beauchamp & Anderson, 2010). Social cognition is defined as the ability to perceive, and process social signals, and to adequately react in social interactions (Crick & Dodge,1994). These social processing skills are largely independent of other cognitive abilities, such as language, intelligence, and attention (Pinkham et al., 2003). A recent review of the scarce literature on social cognitive abilities in children with SCT (Urbanus et al., 2019) suggests that (although these abilities are not yet fully matured), the development of social cognition, assessed by parent-report and performance-based tests, is already found to be affected from age eight years and older. However, social cognitive functioning was not studied in younger age groups.

Social cognitive functioning results from the dynamic and complex development of brain functions and networks in the first years of life. Depending on genetic factors such as SCT, and environmental influences, brain areas involved in perceiving and understanding social information mature, and facilitate social cognitive development. Difficulties with social cognition impact how children perceive and interact with their environment, which is affected in a broad range of psychopathology, including Autism Spectrum Disorder (ASD), and Attention-Deficit/Hyperactivity Disorder (ADHD); children with SCT show higher percentages of these behavior classifications, compared to their peers (for a review see: Van Rijn, 2019). To gain more insight in to the brain-behavior dynamics leading to psychopathology, it is important to investigate age dependent risk factors during the early development of social cognitive skills. Since social impairments have a great impact on everyday life, an objective study of social cognitive abilities during early development of children with SCT is warranted, and could contribute to the identification of indicators for (preventive) support and treatment.

Social situations are rich in providing large amounts of information that need to be processed simultaneously. These situations trigger social cognitive mechanisms in individuals to select information to be able to respond adequately. Central to this selective cognitive processing of social relevant stimuli is the automatic and spontaneous visual orientation towards social information, which is referred to as

social attention. Faces are especially important in the social context, as they provide a wealth of socially relevant information, and are therefore important in successful social interactions and adaptive functioning. Already from birth, newborns show an automatic orientation to faces, and highly prefer to attend to face-like patterns (Johnson, 2005b; for a review on eye tracking studies, see Reynolds & Roth, 2018). Studies have shown that eyetracking is a suitable technique to assess developmental changes in different aspects of visual orientation to social important information in young children. Eye gaze to social information, as measured with eyetracking, is found to be strongly related to the ability to learn from social signals and to develop everyday social behavior (Frank et al., 2014). Even more than other facial characteristics, the eye region is the source of information most used to understand the mental and emotional states of others, and to which we most attend (Itier & Batty, 2009). In young children (and people in general) the preference to visually orient to social stimuli is largely automatic, and requires little effort (Langton et al., 2000). However, the conscious recognition of emotions on faces of others needs more processing time and other higher-order (neuro)cognitive skills are involved (such as language abilities; Adolphs, 2001). The recognition of affective facial expressions gives the opportunity to detect the emotional states of others, and is therefore important during social interactions (Grossmann & Johnson, 2007). It is believed that impairments in social cognition (such as spontaneous visual eye gaze toward social cues and face affect recognition) may be one of the key mechanisms underlying social behavioral difficulties found in individuals with SCT (e.g. Van Rijn, 2019).

Indeed, there is evidence that individuals with SCT attend in a different way to social cues, as compared to individuals without SCT. Eyetracking research in adult men with 47,XXY, and in boys and girls with an extra X chromosome (47,XXX and 47,XXY) showed shorter fixation durations to eyes as compared to boys and girls without an extra X chromosome, and no typical tendency to first fixate on the eyes, both during the scanning of static facial expressions (Van Rijn, 2015), and during dynamic presentation of faces in movie clips (Van Rijn et al., 2014b). Studies also show that boys and adults with 47,XXY have difficulty with the recognition of facial emotions (Samango-Sprouse et al., 2018; Van Rijn et al., 2018). School-aged children and adolescents with an extra X chromosome (47,XXX; 47,XXY) also showed impairments in identifying angry facial expressions (Van Rijn et al., 2014b). However,

to the best of our knowledge, there have been no studies investigating whether these different processes of eye gaze to facial social information and impairments in the recognition of facial expressions also exist in very young children with SCT, and are also present in individuals with 47,XYY. For that reason, the main question of the present study is whether difficulties with eye gaze to faces and affect recognition are already present very early in life. We examined three different age groups within the 1-7 year-old age range. The reason for this was twofold. First, early childhood is a period of rapid maturation of social development at both neural, neurocognitive and behavioral level (Soto-Icaza, Aboitiz & Billeke, 2015). For clinical practice it is therefore important to investigate at what point in early childhood development proceeds differently in children with SCT as compared to peers. It is therefore crucial to study vulnerabilities in different phases of early childhood leading to risk for compromised social development in order to identify early markers for risk and targets for monitoring and intervention. Secondly, in typical development the maturation of social skills is not linear, that is, these social cognitive abilities develop gradually and are intertwined in a temporal sequence of social milestones that may be needed to shape appropriate social functioning (Soto-Icaza, Aboitiz & Billeke, 2015). It is therefore important to focus on vulnerabilities in gaze towards faces and emotion recognition in different phases of early development.

Studies of reduced and deferred eye gaze towards key social emotional features in young children with other genetic syndromes, as compared to children without genetic variations (e.g. fragile X syndrome; Farzin et al., 2009) and ASD (for reviews: Guillon et al., 2014; Chita-Tegmark, 2016) suggest that differences with typically developing groups in processing social cues are partially determined by the nature of the task stimuli. In order to assess the nuances of eye gaze to faces in the current study, three considerations were taken into account while constructing the eye tracking paradigms. First, we studied various outcome measures: the basic ability to gaze to faces, and the choice of focal area when presented with faces for a longer period of time. Second, we studied eye gaze to faces in both static and dynamic paradigms, since it was found that individuals at risk of showing impairments with social attention perform relatively well compared to typically developing peers in tasks that use only static social stimuli, contrasted to tasks with dynamic social stimuli (see for example Freeth et al., 2010). Last, we used paradigms with both single and multiple faces, as it has been found that social content and richness of

the stimuli are significant predictors of social attention difficulties and severity of impairments in social adaptation and communication (see for example: Speer et al., 2007).

To summarize, school-age children, adolescents and adults with SCT are at risk of developing difficulties in social cognitive abilities. More specifically, they show differences in directing their eye gaze to socially important cues as compared to individuals without SCT, and impairments in the recognition of facial affect expressions. Unfortunately, studies investigating the early onset and development of these parameters in very young children with SCT do not exist. A thorough investigation of eye gaze to faces and facial affect recognition skills during different age phases of early development could give more insight in the early markers and developmental pathways leading to social and communication difficulties later in life, and has the potential to provide targets for (preventive) support or intervention.

In this study, we aimed to study these early markers important in social adaptive development. Our research questions were: First, do children in with SCT show differences with processing social information as compared to children without SCT, i.e. attend less to socially relevant cues when looking at static faces, and dynamic social scenes in different age phases of early development? Second, do young children with SCT in different age phases have difficulties with affect recognition skills compared to their typically developing peers? Lastly, we aimed to investigated the role of research site, recruitment bias, and the role of karyotype on eye gaze to faces and affect recognition. Although many factors are involved in presentation of the SCT phenotype, such as timing of diagnosis, the aim of the current study was to contribute to the understanding of the early phenotype of SCT by focusing on eye gaze towards faces and affect recognition in different age groups, which has remained unexplored so far. Based on the relevance of the X and Y chromosomes for development of neural networks supportive of the development of social cognition, we hypothesized that young children with SCT would show different eye gaze patterns to faces and difficulties with emotion recognition, compared to their typically developing peers.

Methods

Participants

The present study is part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study - Leiden the Netherlands), which includes children with SCT and typically developing children aged 1-8 years. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. A group of 100 children with SCT (range 1-7 years old; Mage= 3.69, SD = 1.91) was included in this study, as well as a population-based group of 98 children without SCT (42 boys; M_{age}= 3.66, SD = 1.62). Mean age did not significantly differ between groups (t (196) = 0.11, p = .913). The SCT group consisted of 34 girls with 47,XXX (34%), 45 boys with 47,XXY (45%) and 21 boys with 47, XYY (21%). In order to investigate eye gaze to faces and affect recognition outcomes in different developmental stages in early childhood, the participants were divided in three age groups: children one and two years old (n = 61; M_{age} = 1.47 years, SD_{age} = 0.33; 32 SCT (6 47,XXX, 18 47,XXY, 8 47,XYY), 29 without SCT), children three and four years old (n = 83; M_{age} = 3.88, SD_{age} = 0.58; 40 SCT (13 47,XXX, 19 47,XXY, 8 47,XYY), 43 without SCT), and children five, six and seven years old (n = 54; M_{age} = 5.86, SD_{age} = 0.67; 28 SCT (15 47,XXX, 8 47,XXY, 5 47,XYY), 26 without SCT). To test if the frequencies of SCT types differed across age groups, a χ^2 test was conducted, an no differences were observed (χ^2 (4) = 8.40, p = .078).

Recruitment and assessment took place at 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/University of Colorado in the US. Children in the SCT group were recruited with the help of clinical genetics departments (from the Netherlands and Colorado, US), as well as through patient-advocacy groups and social media postings. For the SCT group, recruitment bias was assessed, three subgroups were identified: (1) 'Active prospective follow-up', which included families who were actively followed after prenatal diagnosis (51% 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 (29% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development (20% of the SCT group).

The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed in the study by standard karyotyping. Sixty-seven children were diagnosed prenatally (65.3%; 20 girls with XXX, 32 boys with XXY, 15 boys with XYY), and 33 children postnatally (34.7%; 14 girls with XXX, 13 boys with XXY, 6 boys with XYY). 24 out of 45 boys with 47,XXY received testosterone treatment (53.3%).
Children without SCT were recruited from the western part of the Netherlands, and approached with information brochures about the study. All participants were Dutch (The Netherlands) or English (USA) speaking, had normal or corrected-to-normal vision, and did not have an history of traumatic brain injury. For ethical reasons, children without SCT 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 ~1 in 1000, the risk of having one or more children with SCT in group children without SCT was considered minimal and acceptable.

Eye tracking paradigms

Eye gaze to static faces. The Static Faces paradigm consisted of 16 static photographs of cross-cultural actors with an equal distribution of two facial emotions (happy and angry), and of male and female actors (see Figure 1). The photographs were taken from the Karolinska Directed Emotional Faces (KDEF; (Lundqvist et al., 1998). These KDEF pictures have no background, and actors have no visible beards, mustaches, earrings, eyeglasses, or make-up. The photographs with 7.99◦ x 10.87◦ visual angle were presented to the child, displayed at the center of the screen, in a counterbalanced order. The child was exposed to each picture for 3 s., with a 2 s. inter-item interval during which a attention grabber (i.e. a picture of a toy or animal, together with a sound to grab the child's attention) was presented in one of the four corners of the screen, to prevent for the automatic response to fixate at the center of the screen.

Eye gaze patterns to single and multiple faces. The Dynamic Social Information eyetracking paradigm consisted of two natural and dynamic conditions: single face (SF) and multiple faces (MF). Six trials were included (3 single face, 3 multiple faces) of 15 s. each. The total time of the stimulus set was 90 s. The trials with 16.98◦ x 29.73◦ visual angle were presented in an alternate order (i.e. single, multiple, single, multiple, single, multiple). In each trial, a video clip was presented to the child. In

the single face condition, one face of a child was on the screen; in the multiple faces condition, two or more faces were on the screen (child-child, child-adult or child-adult-adult). The video clips consisted of subjects with different cultural backgrounds, and were extracted from the TV broadcasted series 'Baby Einstein' (Kids2, 2015; see Figure 1). The videos were accompanied by unsynchronized classical instrumental music, and no speech was involved. As these eyetracking paradimgs did not involve language and used age-appropriate stimuli, it was considered to be appropriate for participants in both countries. In a group of non-clinical young children aged 3-7 years, this eye tracking paradigm was found to be significantly predictive of real-life social behaviors, and independent of age, IQ, or gender (Van Rijn et al., 2019).

Figure 1. Examples of photographs in the Static Faces paradigm: (1) happy face, (2) angry face (taken from KDEF; Lundqvist et al. 1998); and screenshots of videoclips in the Dynamic social information paradigm: (3) single face, (4) multiple faces.

Eye tracking equipment and procedures

Gaze data within specific areas of interest (AOIs) was 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 15.6" computer screen with 1920 x 1080 resolution (visual angle = 16.98◦ x 29.73◦) with eye tracker was placed on a table adapted to the height of the seat, and the child was seated in a car seat at 65 cm viewing distance which is within the ideal range for recording, according to the Tobii X2-60 manual. A 5-point calibration procedure was used, with successful calibration defined as a maximum calibration error of 1◦ for individual calibration points (i.e. < 1 cm at a distance of 65 cm from the eyetracker). After the calibration procedure, the child was instructed to watch the movie clips and pictures on the computer. The two eye tracking paradigms started with an attention grabber (e.g. a moving picture of an animal, shown on a black background and accompanied by a sound) to direct the attention of the child to the screen.

Gaze data was processed using Tobii Studio (version 3.2.1), using the Tobii Identification by Velocity Threshold (I-VT) fixation filter. This filter controls for validity of the raw eyetracking data making sure only valid data were used (Olsen, 2012). The I-VT Threshold filter was set to define the minimum fixation duration to 60 ms, with a velocity threshold of 30◦/s. Data were considered valid and were included in analysis if one or both eyes had a valid reading according to the Tobii validity criteria.

The 'Dynamic AOI' tool was used to draw AOIs, drawn with a one centimeter margin, to ensure that the AOIs were sufficiently large outside the defining contours to reliably capture the gaze fixation (Hessels et al., 2016). In the Static Faces paradigm, AOIs were grouped into the category eyes (visual angle = 5.28◦ x 1.75◦) and for the whole screen (visual angle = 16.98◦ x 29.73◦); first fixations within the eye AOI, and total fixation duration within the eye AOI were measured, in order to study eye gaze to eyes. In the Dynamic Social Information paradigm, dynamic AOIs were grouped into the following categories: face and eyes, and for the whole screen (visual angle = 16.98◦ x 29.86◦). Total fixation duration within AOIs were measured in two conditions: Single Face condition and Multiple Face condition. In order to evaluate the amount of nonvalid eye tracking data, the total visit duration toward the whole

screen was calculated, divided by the duration of the clip, multiplied by 100, reflecting the percentage of valid data collected during each of the eye tracking tests. For both paradigms, proportions fixation duration were calculated by taking the total fixation duration within the AOI, divided by the total visit duration toward the whole screen of the individual child, multiplied by 100, reflecting the percentage of time children were attending to an AOI. In the facial emotion paradigm, proportions first fixations within the AOI eyes were calculated by taking the number of photographs where participants fixated first on the eyes, divided by the total number of photographs (max = 16).

NEPSY Affect recognition

The Affect Recognition subtest of the Developmental NEuroPSYchological Assessment, second edition (NEPSY-II neuropsychological test battery; Korkman et al., 2007) was designed to assess children's ability to discriminate among common facial emotions from photographs of children, and used in this study to measure task performance of affect recognition skills. The task has been normed with typically developing children aged 3-16 years old, and was administrated in a subgroup of the study sample with the age of 3 years and older (n = 138). During the task, participants are required to match faces of different children with different cultural backgrounds who show the same emotional expressions (happy, sad, angry, disgust, fear and neutral). The participant indicates if two expressions are the same or different, determines which two faces have similar expressions, or identifies two children with expressions that match a third child's face. The total raw score range is between 1 and 25, with higher scores reflecting a better ability to recognize facial expressions. Besides raw scores, percentile scores as compared to norms from the general population can be calculated. Dependent upon the spoken language of the child, the Dutch or English norms were used. Percentile scores were labeled as being in the average range (percentile score > 25), the borderline range (11 < percentile score > 25), the below expected level (3 < percentile score > 10), and the well below expected level (percentile score ≤ 2).

Cognitive assessment

To measure global level of intelligence and language three tests were administrated.

The Bayley-III (subscale cognitive scale; Bayley-III: Bayley Scales of infant and toddler development, 2009) was administered to children with the age of 1-2 years old. In the older children four subtests of the Wechsler Preschool and Primary Scales of Intelligence, 3rd edition (WPPSI-III) were used to estimate global level of intelligence (children aged 3 years: Block Design, Receptive Vocabulary, Information, Object Assembly; children aged 4 years and older: Block Design, Matrix Reasoning, Vocabulary, and Similarities; (Wechsler, 2002). For children aged 4 years and older, Total IQ estimates were calculated based on this short form version of the WPPSI-III (Hurks et al., 2016). The Peabody Picture Vocabulary Test (PPVT-III; Dunn & Dunn, 1997) was used to measure receptive language level in children aged 3 years and older.

Study procedures

Assessment took place at various sites (Colorado USA and the Netherlands) either in a quiet room at the University (lab assessment) or at home (home assessment). To standardize the testing environment, the testing set-up and research protocols were identical at 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). Administration of cognitive and language assessment and the NEPSY was performed on a table by trained child psychologists or psychometrists in Dutch or English (dependent on the first language of the child). The eye tracking procedure took place during a separate appointment, within one week after the NEPSY administration. The laptop with the eye tracker was placed in a small tent to standardize the testing environment, and to control for lighting conditions. The child was seated in a car seat in front of the eye tracker. The examiner was seated beside the child (directing Tobii Studio with a remote keyboard) and started the calibration procedure. Eye tracking paradigms were shown in a fixed order (single/multiple faces, static faces). Parents were allowed to stay in the room (out of sight) and were asked not to communicate with their child during the procedure.

Data analyses

Statistical Package for the Social Science (SPSS) version 25 was used for statistical analyses. A χ^2 test was used to compare the distribution of karyotypes within the three

age groups. Pearson's correlation analyses were used to measure the association between main outcome variables (i.e. eye gaze to faces and affect recognition) and global cognitive functioning and receptive language abilities. For group wise (SCT vs. children without SCT) comparisons of proportions first fixations, proportions duration fixation within the AOIs in the three age groups, and affect recognition skills in two age groups (M)ANOVAs were used. Pillai's trace was used to assess the multivariate effect. Significant multivariate effects were post-hoc analyzed with univariate ANOVAs to determine the locus of the multivariate effect. Influence of karyotype accounting for the effect of age was tested by an MANCOVA. (M)ANOVAs were used to investigate differences between recruitment groups, and influence of research sites was analyzed with independent t-tests. Statistical analyses were performed one-tailed (SCT vs. children without SCT) or two-tailed (influence of karyotype/recruitment bias/research site), and level of significance was set at $p < .05$. In case of significant differences, Cohen's d or partial η^2 were used to calculate effect sizes.

Results

Eyetracking: data quality

Eye gaze to static faces. The Static Faces paradigm was successfully completed by 181 children (18 children were not able to complete the task due to technical issues or fatigue of the child). Valid data were ensured by screening attention to the screen on a stimulus to stimulus basis, and stimuli of <30% attended were omitted from calculation of the average looking time for each individual child. After screening, the total proportion valid on-screen visit duration (averaged across conditions) was 83.3% and did not significantly differ between children with and without SCT, t (179) = -1.10, p = .272. Proportion first fixation and proportion fixation duration to eyes were not correlated to global cognitive functioning (respectively: r = .119, p = .114; r = .143, p = .058), and were not different between lab and home assessments (respectively: t (179) = -1.83, p = .069; t (179) = -.60, p = .549). See Table 1 for descriptive statistics for all outcome measures in the SCT and typically developing group.

Eye gaze patterns to single and multiple faces. The Dynamic Social Information paradigm was successfully completed by 188 children (11 children were not able to complete the task due to technical issues or fatigue of the child). Total proportion

Table 1. Descriptive statistics for SCT and TD group.

	Age group	*N*	Missing	Condition	% valid data	SCT		TD		F (df), *p* and effect size
						Min-Max	*M (SD)*	Min-Max	*M (SD)*	
Cognitive development *Norm score; Bayley-III*	1-2 years	60	1			80-125	100.39 (12.18)	72-129	99.48 (14.32)	$F(1,58) = 0.70$, $p = .793$; $\eta_p^2 = .01$
Total IQ *WPPSI-III*	3-7 years	131	7			55-138	95.48 (19.88)	72-140	109.01 (13.27)	$F(1,129) = -21.37$, $p < .001$; $\eta_p^2 = .14$
Receptive language *Standard score; PPVT-III*	3-7 years	135	3			65-129	99.26 (14.94)	74-133	108.67 (12.44)	$F(1,133) = -15.86$, $p < .001$; $\eta_p^2 = .11$
Eye gaze to static faces *Proportions first fixations*	1-7 years	181	18		83.3% (angry condition: 78.5%; happy condition: 88.1%)	.00 - 1.00	.58 (.24)	.00 - 1.00	.63 (.27)	$F(1,179) = -1.83$, $p = .089$; $\eta_p^2 = .01$
Eye gaze to static faces *Proportions fixation duration*	1-7 years	181	18			.00 - .69	.32 (.17)	.00 - .73	.35 (.20)	$F(1,177) = -0.81$, $p = .185$; $\eta_p^2 = .01$
Eye gaze patterns to single and multiple faces *Proportions fixation duration*	1-7 years	188	11	SF: face	85.3%	.01-.79	.46 (.17)	.04 - .83	.51 (.19)	$F(1,186) = -4.66$, $p = .016$; $\eta_p^2 = .02$
				SF: eyes		.00 - .59	.21 (.13)	.00 -.61	.23 (.15)	$F(1,186) = -1.48$, $p = .113$; $\eta_p^2 = .01$
				MF: faces	81.5%	.04 - .79	.51 (.16)	.04 -.80	.57 (.19)	$F(1,186) = -5.35$, $p = .011$; $\eta_p^2 = .03$
				MF: eyes		.00 - .45	.14 (.11)	.00 - .57	.18 (.13)	$F(1,186) = -4.56$, $p = .017$; $\eta_p^2 = .023$
Affect recognition *Raw score; NEPSY-II*	3-7 years	130	8			1-23	12.07 (4.58)	1-23	13.64 (4.86)	$F(1,128) = -3.58$, $p = .031$; $\eta_p^2 = .03$

Note: SCT = Sex Chromosome Trisomy; TD = Typically Developing; SF = single face; MF = multiple faces.

valid on-screen visit duration (averaged across conditions) was 83.4% and did not significantly differ between children with and without SCT, t (186) = -0.10, p = .921. Proportion fixation duration to eyes and faces in the Single Face condition were not correlated to global cognitive functioning (AOI face in Single Face condition: r = .062, p = .403; AOI eyes in Single Face condition: r = .104, p = .161) Similar, proportion fixation duration to faces in Multiple Faces condition was not correlated with global cognitive functioning: r = .111, p = .135). However, proportion fixation duration to eyes in the Multiple Faces condition was related to global intellectual functioning, r = .169, p = .022. Outcomes measures were not different between lab and home assessments (SF faces: t (186) = 0.53, p = .594; SF eyes: t (179) = -.0.77, p = .445; MF faces: t (179) = -.036, p = .723; MF eyes: t (179) = -1.75, p = .081. See Table 1 for descriptive statistics for all outcome measures in the SCT and typically developing group.

Eye gaze to static faces: age dependent group differences

Proportions of first fixations on eyes. Age dependent SCT vs. typically developing group differences in first tendency to look at eyes were analyzed, when presented with static photographs of faces. Three separate ANOVAs in the three age groups were carried out with two groups (SCT vs. children without SCT) on proportions of faces where participants first fixated on the eyes. No significant effects of group (SCT vs. children without SCT) were found in the 1-2 years-old group (F (1,49 = 0.169, p = .342), and the 3-5 years old group (F (1,74) = 0.479, p = .246). A borderline group effect (SCT vs. children without SCT) was found in the 5-7 years old group (F (1,52) = 2.288, p = .068). See Table 2 for M, and SDs.

Table 2. Eye gaze to static faces (proportions first fixations; proportions fixation durations) in three age groups.

		Phases of development										
		1, 2 years old (n = 51: 24 SCT, 27 TD)			**3, 4 years old** (n = 76: 34 SCT, 42 TD)			**5, 6, 7 years old** (n = 54: 28 SCT, 26 TD)				
Eye gaze to static faces	**AOI**	SCT *M* *(SD*	TD *M* *(SD)*	*p*-value	SCT *M* *(SD)*	TD *M* *(SD)*	*p*-value	SCT *M* *(SD)*	TD *M* *(SD)*	*p*-value	Post-hoc effect	Effect size (η_p^2)
Proportion first fixation	Eyes	.51 (.26)	.53 (.26)	.342	.60 (.24)	.65 (.29)	.246	.62 (.23)	.71 (.22)	.068		
Proportion fixation durations	Eyes	.35 (.19)	.30 (.22)	.192	.35 (.17)	.38 (.20)	.289	.28 (.14)	.38 (.17)	.016	SCT < TD	.09

Note: SCT = Sex Chromosome Trisomies; TD = Typically Developing.

Proportions of fixations duration on eyes. Age dependent SCT vs. typically developing group differences in eye gaze to faces were analyzed, when presented with static faces: three separate ANOVAs with two groups (SCT vs. children without SCT) were carried out on proportions of fixation duration to eyes. In the 1-2 years-old age group, no significant effect of group (SCT vs. children without SCT) was found on the proportions of fixation duration, F (1,49) = 0.771, p = .192. Also, in the 3-5 years-olds, no significant effect of group (SCT vs. children without SCT) was found on the proportions of fixation duration, F (1,74) = 0.314, p = .289. However, in the 5-7 year-olds, a significant effect of group (SCT vs. children without SCT) was found on the proportions of fixations duration for the AOI eyes (F (1,51) = 4.925, p = .016, η_p^2= .09): the SCT group spent less time fixating on eyes, compared to their typically developing peers. See Table 2 for *M* and *SDs*.

Eye gaze patterns to single and multiple faces: age dependent group differences

Proportions of fixation duration on eyes and faces. Within each age group, differences in eye gaze to faces with one single face (Single Face condition) and multiple faces (Multiple Faces condition) were analyzed with three separate MANOVAs, using Pillai's trace. Descriptive statistics can be found in Table 3. In the 1-2 year-olds, there was no significant effect of group (SCT vs. children without SCT) on the proportions of fixation duration for the AOIs in both the SF and MF condition, F (4,52) = 0.439, p = .390. In the 3-5 year-old age group, a significant effect of group (SCT vs. children without SCT) was found, F (4,72) = 2.782, p = .017, η_p^2 = .13. Post-hoc ANOVA tests on the outcome variables revealed a significant group effect with a medium effect size on the proportions of fixation duration for AOI face in the SF condition such that the SCT group spent less time fixating on the face when compared to their typically developing peers. In the 5-7 year-olds, a significant effect of group was found (SCT vs. children without SCT), F (4,49) = 2.165, p = .044, η_p^2= .15. Post-hoc ANOVA tests on the outcome variables revealed significant group effects on the proportions of fixation duration for AOI face and AOI eyes in the MF condition with a medium effect size, revealing that the SCT group spent less time fixating on faces and eyes, when compared to children without SCT.

Table 3. Eye gaze patterns to single and multiple faces: proportions fixation duration on eyes and faces in two conditions (single face, multiples face): outcomes in three age groups and moderated effect of age.

		Phases of development											
		1,2 years old (N = 57: 30 SCT, 27 TD)			**3,4 years old** (N = 77: 35 SCT, 42 TD)				**5,6,7 years old** (N = 54: 28 SCT, 26 TD)				
	AOI	SCT *M (SD)*	TD *M (SD)*	*p*-value	SCT *M (SD)*	TD *M (SD)*	*p*-value	Post-hoc effect	Effect size (part. η^2)	SCT *M (SD)*	TD *M (SD)*	*p*-value	Post-hoc effect	Effect size (η_p^2)
Single Face	**Faces**	.52 (.17)	.57 (.21)	.132	.45 (.16)	.54 (.16)	.009	SCT < TD	.07	.40 (.16)	.40 (.15)	.458		
	Eyes	.20 (.14)	.20 (.16)	.474	.22 (.14)	.26 (.15)	.142			.20 (.12)	.22 (.10)	.225		
Multiple Faces	**Faces**	.56 (.15)	.60 (.21)	.172	.53 (.14)	.57 (.18)	.124			.43 (.18)	.52 (.17)	.029	SCT < TD	.07
	Eyes	.13 (.11)	.13 (.13)	.437	.16 (.12)	.20 (.13)	.074			.14 (.09)	.19 (.13)	.033	SCT < TD	.06

Note: SCT = Sex Chromosome Trisomies; TD = Typically Developing; AOI = Area of Interest

Facial Affect recognition: age dependent group differences

The NEPSY Affection recognition task was administered only in the group of children aged 3 years and older (n = 138). Eight children were not able to finish the NEPSY Affect recognition task (total n = 130; 61 SCT (26 children with 47, XXX; 26 children with 47, XXY; 9 children with 47,XYY), 69 without SCT). Affect recognition scores were not correlated to global cognitive functioning (r = .162, p = .071), but were correlated to receptive language skills (r = .604, p <.001). See Table 1 for descriptive statistics of all outcome variables for both the SCT and typically developing group.

Within the two age groups (3-5; 5-7), differences in affect recognition were analyzed with two separate ANOVAs. Differences between the SCT group and their typically developing peers were found in both age group, see Figure 2. When evaluating scores normalized for age, for affect recognition in the SCT group 54.2% scored in the average level, 5.1% in the borderline range, 8.5% scored in the below expected level, and 32.2% in the well below expected level.

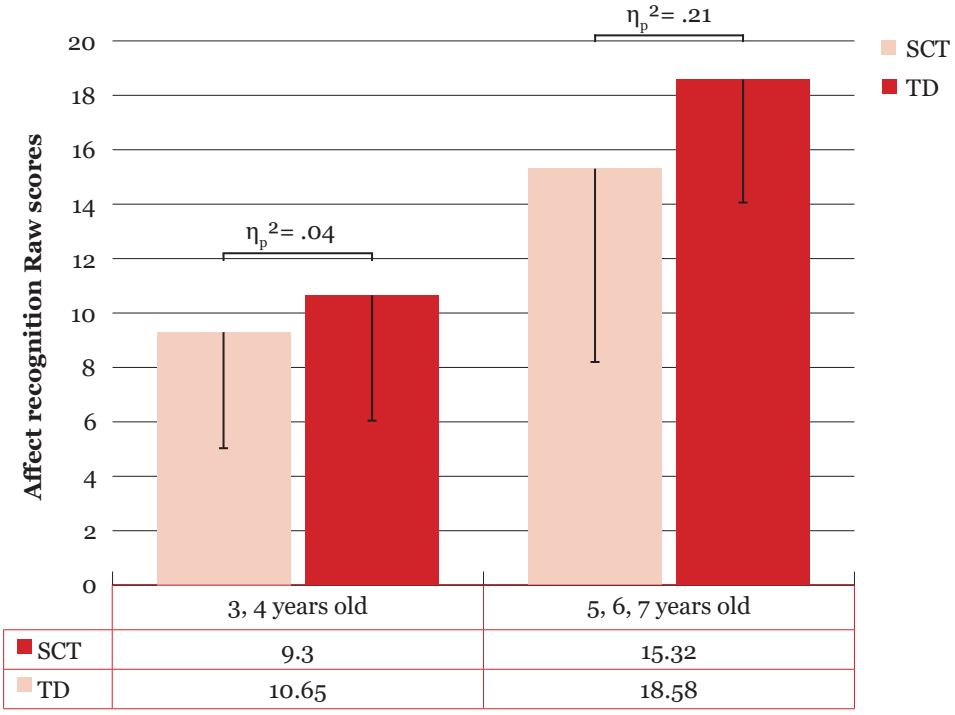

Figure 2. Affect recognition in SCT vs. TD group and age groups.
Note: SCT = Sex Chromosome Trisomies; TD = Typically Developing. η_p^2= effect size; ⟝ = Standard Deviation (only lower bar depicted)

Karyotype differences within the SCT group

In order to investigate the influence of various karyotypes on eye gaze to faces and affect recognition taking into account the effect of age, MANCOVAs were carried out with main effect of karyotype (XXX vs. XXY vs. XYY), and age as covariate. No differences between karyotypes were found for all eyetracking outcome measures. A significant difference between karyotypes was found for affect recognition (XXY < XXX), when age was accounted for and kept constant. See Table 4 for Estimated Marginal Means, and *p*-values, post-hoc effects and effect sizes.

Recruitment bias within the SCT group

Within the SCT group we tested with MANOVA for differences on eye gaze to faces and ANOVA for difference on affect recognition between the three recruitment groups (A: prospective follow-up, B: information seeking parents, and C: clinically referred cases group). Differences between recruitment groups were only analyzed in the study measures in which a difference was found between children with and without SCT. There were no significant differences for study outcomes between the recruitment groups (except for proportions fixation duration on faces in the dynamic social information paradigm; single faces). See Table 5 for means, exact *p*-values, post-hoc effects and effect sizes.

Table 4. Differences between karyotypes on eye gaze to faces and affect recognition accounted for age (EMM (SE)).

			47,XXX	47,XXY	47,XYY	*p*-value	*p*-value	Effect size (η_p^2)
Eye gaze to faces	**Condition**	**AOI**	*n* = 30	*n* = 41	*n* = 15			
Eye gaze to static faces *Proportions first fixations*		Eyes	.60 (.05)	.69 (.05)	.49 (.08)	.074		
Eye gaze to static faces *Proportions fixation duration*		Eyes	.29 (.03)	.40 (.03)	.30 (.05)	.056		
Eye gaze patterns to single and multiple faces *Proportions fixation duration*	Single Faces	Faces	.46 (.03)	.45 (.03)	.40 (.05)	.547		
		Eyes	.22 (.03)	.25 (.03)	.17 (.04)	.284		
	Multiple Faces	Faces	.51 (.03)	.51 (.03)	.40 (.06)	.223		
		Eyes	.17 (.02)	.18 (.02)	.09 (.04)	.107		
NEPSY Affect Recognition			*n* = 28	*n* = 19	*n* = 14			
Raw score			13.56 (.67)	10.55 (.69)	11.98 (1.12)	.011	XXY<XXX	.14

Note: EMM = Estimated Marginal Means; AOI = Area of Interest.

Table 5. Differences in eye gazes to faces and affect recognition across SCT recruitment groups (M, SD).

			Prospective follow-up (A)	Information seeking parents (B)	Clinically referred cases (C)	*p*-value	Post-hoc effect	Effect size (η_p^2)
Eye gaze to faces	**Condition**	**AOI**	*n* = 41	*n* = 25	*n* = 18			
Eye gaze to static faces *Proportions fixation duration*		Eyes	.32 (.17)	.35 (.19	.33 (.15)	.851		
Eye gaze patterns to single and multiple face *Proportions fixation duration*	Single Faces	Faces	.42 (.17)	.52 (.15)	.44 (.14)	.032	A < B	0.12
	Multiple Faces	Faces	.47 (.18)	.57 (.14)	.51 (.14)	.080		
		Eyes	.12 (.11)	.18 (.12)	.16 (.10)	.117		
Affect Recognition			*n* = 28	*n* = 19	*n* = 14			
Raw scores			11.14 (4.55)	12.21 (4.69)	13.71 (4.30)	.229		

Note: SCT = Sex Chromosome Trisomy; AOI = Area of Interest.

The role of research site

To control for the potential impact of research site on outcomes of the study, the data of the two research sites were compared. Comparing the outcome measures in the SCT group between both research sites (the Netherlands vs. US), revealed a consistent pattern of results, indicating that none of the eye movement measures showed significant differences between research sites (see Table 6). However, a significant difference between research sites was found for affect recognition skills: children in the USA had lower affect recognition scores (M=10.65, SD=3.90), compared to children in the Netherlands (M=13.11, SD=4.81; p =.037, Cohen's d = 0.56).

Table 6. Impact of research site on eye gaze to faces and affect recognition in the SCT group.

	Condition	The Netherlands *M (SD)*	USA *M (SD)*	*t*-value	*p*-value
Eye gaze to static faces *Proportions first fixations*		.61 (.22)	.55 (.26)	1.05	.296
Eye gaze to static faces *Proportions fixation duration*		.32 (.16)	.32 (.19)	0.14	.887
Eye gaze patterns to single and multiple faces *Proportions fixation duration*	SF: face	.43 (.16)	.48 (.17)	1.46	.147
	SF: eyes	.21 (.13)	.20 (.13)	0.22	.823
	MF: faces	.50 (.18)	.52 (.15)	0.61	.545
	MF: eyes	.15 (.11)	.13 (.10)	0.92	.361
Affect Recognition *Raw scores*		13.11 (.4.81)	10.65 (3.90)	2.14	.037

Discussion

This study aimed to investigate age dependent eye gaze to faces and affect recognition vulnerabilities in very young children with sex chromosome trisomies (SCT) aged 1-7 years. Key outcomes of the study include differences in automatically orienting and holding eye gaze to socially important information between children with and without SCT, suggesting that young children with SCT are less inclined to automatically orient towards social information. These difficulties with directing eye

gaze to social important information were most pronounced in children aged three years and older, and when the richness of the social stimuli was high (i.e. multiple faces). Also affect recognition impairments were found, with on average 32.2% of the group children with SCT scored in the well below expected range.

First of all, we explored with the help of eyetracking measures gaze to static faces, and gaze patterns to eyes and faces in dynamic social scenes with a single face and multiple faces. Analyses in specific age groups revealed that 1-2 years old children with SCT showed no different tendency to initially fixate on the eyes, when presented with a face. Also, when presented with dynamic social interactions, the results revealed no differences in eye gaze patterns between 1-2 years-olds with and without SCT (i.e. displayed no shorter fixation duration to eyes nor to face). However, in the 3-5 year-old group we did find differences between the children with SCT and typically developing children: children with SCT were less inclined to fixate eye gaze towards faces when looking at dynamic social stimuli with a single face, although percentages of first fixation to the eye region of static faces were similar to 3-5 year-olds without SCT. These results suggest that children with SCT aged 3-5 years assess static faces with emotions as fast as their typically developing peers, but are less consistent in their choice of focal area when presented with dynamic social stimuli with one single face.

Moreover, 5-7 year-olds with SCT showed lower fixation duration on eyes compared to typically developing children, when presented with static faces. In addition, children with SCT aged 5-7 years-old fixated less on socially important information (both faces and eyes) when presented with dynamic social stimuli with multiple faces. This pattern of findings among 5-7 year-olds shows that differences in eye gaze to social stimuli between the SCT and typically developing group occur as a function of the richness of social information: if the richness of socially relevant and dynamic information is high (i.e. multiple faces), children with SCT deviate eye gaze from central and important social information (i.e. eyes and faces).

Taken together, these eyetracking results reveal that children with SCT generally are less inclined to automatically orient their gaze at relevant social-emotional information (i.e. eyes and faces), compared to typically developing children from preschool age on. Research has shown that typically developing children

preferentially attend to social stimuli, beginning as early as infancy. Furthermore, high social content typically increases attention of children towards the eyes and faces (Birmingham et al., 2008). However, our results suggest that, on average, very young children with SCT, on average, have difficulties with attention to social cues, with more impairments when presented with high richness of social information (i.e. multiple faces) as compared to on single faces, and more impairments in children of older age, as compared with their typically developing peers.

Reduced eye gaze to socially meaningful and complex stimuli already during early development, and more pronounced difficulties with eye gaze to social relevant stimuli if the amount of social information is rich, may have substantial impact on the fundamentals of social learning. Reduced eye gaze to social important information may lead to limited quantitative and qualitative opportunities to acquire social knowledge in children with SCT, and to learn from (complex) social interactions (Mundy & Neal, 2001). Attending to another person's face and eyes allows typically developing children to have rich social experiences that are crucial for the development of social and communicative abilities, such as joint attention, language acquisition, and face or affect recognition (Gliga & Csibra, 2007). Consequently, avoidance of the eyes and faces of others may have a broad impact on the complex maturation of social (cognitive) abilities, which are built upon basic social-perceptual information. Earlier studies reported social attention deficits in adult men with an extra X chromosome (Van Rijn et al., 2014b; Van Rijn, 2015). These adult studies might represent the cumulative effects of long-term atypical visual orientation to socially important information, whereas the results of the current study suggest a developmental pathway in which profiles of impairments are emerging during early childhood.

Although such longitudinal relations between eye gaze to social important information and more complex social processing abilities not being assessed in this study, we did investigate age dependent affect recognition skills in very young children with SCT, between the ages of 3-7 years. A difference was found for affect recognition abilities between children with and without SCT from the age of 3 years old, indicating deficits in young children with SCT. Earlier studies also found impairments with affect recognition in school-aged children and adolescents with SCT, in both parent report of individuals with SCT (Ross et al., 2012; Cordeiro et al., 2012; Van Rijn et

al., 2014a) and direct assessment of individuals with an extra X chromosome (Van Rijn et al., 2018; Samango-Sprouse et al., 2018; Van Rijn et al., 2014a). Percentages of young children with SCT that scored in the clinical range in the current study (32.8%) are comparable with earlier research in older individuals with an extra X chromosome, and add to the literature that clinically significant deficits already arise early in development, and can also be found in boys with 47,XYY.

A difference between research sites was found for affect recognition abilities in children with SCT, which may suggest that cultural and social factors can be related to emotional processing. Although we acknowledge that cultural differences may contribute to some of the variance in the outcome, we are confident that this is not relevant for the systematic group differences between the SCT and typically developing group that we found in the current study. Further research could study the influence of ethnicity, cultural differences, and family environment on affect recognition abilities in children with SCT. As no differences between research sites were found for study outcomes measured with eyetracking methods, we suggest to use eyetracking methods in international studies aimed to measure the influence of culture on emotion processing.

When exploring the influence of specific karyotype (XXX, XXY, XYY) on eye gaze patterns to faces and affect recognition, accounting for the effect of age, results showed that for the majority of social cognitive measures no significant differences between the karyotypes were found. However, for affect recognition boys with 47,XXY showed the be more vulnerable as compared to some of the other SCT karyotypes (see Table 5). These results suggest that although eye gaze patterns to faces and affect recognition were impaired in all karyotypes and older children with SCT had more difficulties than younger children, boys with XXY may be more vulnerable in their ability to recognize facial affects than other SCT karyotypes.

The results of the present study have clinical implications. Effects of chromosomal trisomies often become more apparent later on in development, when a child is faced with developmental tasks and when compromised development of the brain leads to an increasing discrepancy with the age-required norms (Rourke et al., 2007; Sprong, 2008). It is therefore important that social attention and affect recognition skills are included in standard neuropsychological assessment from the age of three years

old, in addition to assessments of language and learning difficulties, to allow for close monitoring in children with SCT. Sensitive developmental periods also serve as key windows of opportunity, and early implementation of (preventive) support and intervention programs on social attention and affect recognition skills have the potential to reduce risk for social and communication impairments, and to optimize quality of life.

Regarding possible bias of recruitment on the outcomes variables, eye gaze patterns to faces and affect recognition (except for one eyetracking parameter) were not dependent on recruitment strategy, i.e. prospective follow-up group, information seeking parents group, or clinically referred cases group. These findings suggests that the outcomes of this study are representative for this group of diagnosed children with SCT as a whole. However, it remains unsure to what degree the findings in this study can be generalized to those who have SCT, but remain undiagnosed (see for example: Berglund et al. (2019) for estimated proportions of underdiagnosing in SCT). This may concern children who do not require clinical care or children who do require care, but for whom it is not known that SCT is an underlying genotype.

Limitations of the current study include the cross-sectional design that limits cause-effect conclusions. Future studies should focus on the longitudinal development of social attention in children with SCT, and the impact of altered attention to social information on affect recognition and other social (cognitive) functions (e.g. Theory of Mind). In this study, we only focused on gaze towards faces with affective expressions of basic emotions, as these convey a high load of social information, more so than neutral faces. Based on our findings, it would be interesting to learn more about the impact of SCT on the scanning of faces in general. Future research should also address the questions whether intervention programs targeting the early development of affect recognition skills are effective in improving these skills and if so, whether interventions lead to improved social behavioral outcomes. As it was beyond the scope of this study to investigate the influence of testosterone treatment in boys with 47,XXY, future studies with suitable designs (e.g. Randomized Control Trials) should study these parameters in relation to general social cognitive functioning in children with SCT.

Conclusion

In conclusion, the overall results of this study indicate that young children with SCT (on average) have difficulties automatically orienting and holding their attention to socially important information, especially when the richness of social stimuli is high (i.e. multiple faces). These difficulties with eye gaze to social stimuli were found in children with SCT aged 3 and older. In addition, impairments in facial affect recognition skills were found, with 32.8% of the SCT children scoring in the clinical range. This calls for a focus on the monitoring of social cognitive functioning from an early age onwards in SCT. These findings also highlight the importance of further exploring the developmental pathway of social attention in children with SCT in studies with a longitudinal design that allows for more understanding of the predictive value of these social cognitive skills for social behavioral difficulties and psychopathology, and the implementation of (preventive) early interventions aiming to support social cognition, to positively influence developmental outcomes in children with SCT.

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CHAPTER 6

Early symptoms of Autism Spectrum Disorder (ASD) in 1-8 years old children with Sex Chromosome Trisomies (XXX, XXY, XYY), and the predictive value of Joint Attention

Bouw, N., Swaab, H., Tartaglia, N., Wilson, R. L., & Van Rijn, S. (2022). Early symptoms of autism spectrum disorder (ASD) in 1–8 year old children with sex chromosome trisomies (XXX, XXY, XYY), and the predictive value of joint attention. *European Child & Adolescent Psychiatry*, 1-12.

Abstract

Objective: The objective of the present study is to investigate the impact of Sex Chromosome Trisomy (SCT; XXX, XXY, XYY) on the early appearance of Autism Spectrum Disorder (ASD) symptoms, and the predictive value of Joint Attention for symptoms of ASD. SCTs are specific genetic conditions that may serve as naturalistic 'at risk' models of neurodevelopment, as they are associated with increased risk for neurobehavioral vulnerabilities.

Methods: A group of 82 children with SCT (aged 1-8 years old) was included at baseline of this longitudinal study. Joint Attention was measured at baseline with structured behavior observations according to the Early Social Communication Scales. ASD symptoms were assessed with the Modified Checklist for Autism in Toddlers questionnaire and Autism Diagnostic Interview-Revised in a one-year follow-up. Recruitment and assessment took place in the Netherlands and in the United States.

Results: The results demonstrate that ASD symptoms were substantially higher in children with SCT compared to the general population, with 22% of our cohort at clinical risk for ASD, especially in the domain of social interaction and communication. Second, a predictive value of Joint Attention was found for ASD symptoms at one year follow-up. In this cohort, no differences were found between karyotype-subtypes.

Conclusions: In conclusion, from a very early age, SCT can be associated with an increased risk for vulnerabilities in adaptive social functioning. These findings show a neurodevelopmental impact of the extra X or Y chromosome on social adaptive development associated with risk for ASD already from early childhood onward. These findings advocate for close monitoring and early (preventive) support, aimed to optimize social development of young children with SCT.

Introduction

Sex Chromosome Trisomies (SCTs) are among the most common chromosomal aneuploidies in humans (Boyd, Loane, Garne, Khoshnood & Dolk, 2011), with a prevalence of 1:650-1:1000 of live births (Berglund, Viuff, Skakkebæk, Chang, Stochholm, & Gravholt, 2019). SCT, the presence of an extra X or Y chromosome, lead to the chromosomal patterns of 47,XXX in girls (Triple/Trisomy X), and 47,XXY (Klinefelter syndrome) and 47,XYY (XYY syndrome) in boys. SCT is characterized by a mild physical phenotype shared across SCT variants, with minimal atypical facial characteristics, tall stature, and low muscle tone (Tartaglia et al., 2020). On social behavioral level, SCT is associated with increased risk for challenges in social adaptive functioning, including shyness, social immaturity, difficulties in forming interpersonal relationships, increased levels of social anxiety, social impulsivity, and impairments in underlying social cognitive mechanisms (see for reviews: Freilinger, Kliegel, Hänig, Oehl-Jaschkowitz, Henn & Meyer, 2018; Tartaglia, Howell, Sutherland, Wilson & Wilson, 2010; Ross et al., 2012; Urbanus, Van Rijn & Swaab, 2020).

The severity of social behavioral vulnerabilities in school-aged children, adolescents and adults with SCT is illustrated by an increased level of symptoms and clinical diagnoses of Autism Spectrum Disorder (ASD; see for a review: Van Rijn, 2019). Previous studies determining the impact of SCT on ASD symptomatology have focused on populations with broad age-ranges, including participants from middle childhood to adulthood. On average across these studies, depending on the ascertainment methods, diagnostic measurements and criteria used, about 15% (range 10.8-20%) of individuals with 47,XXX; 18% (range 10-27%) of individuals with 47,XXY and 30% (range 19-43%) of individuals with 47,XYY meet the full criteria of a clinical diagnosis of ASD (Cederlöf et al., 2014; Ross et al., 2012; Wigby et al., 2016). Thus, compared to a worldwide prevalence rate of ASD of 0.6% in the general population (Elsabbagh et al., 2012), the prevalence of ASD has been shown to be seriously higher in SCT. Even when the most conservative prevalence rate is considered and acknowledging some level of ascertainment bias in previous studies, there is consistent evidence that SCT is associated with a significantly elevated risk for ASD relative to population estimates. However, information on early developmental pathways in young children with SCT before the age of 6 years precursing these social impairments later in life is extremely limited, as shown by a review (Urbanus, Van Rijn & Swaab, 2020). This is

unfortunate, as early childhood is a period in which brain networks that biologically underpin social (cognitive) development rapidly mature and specialize (Atzil, Gao, Fradkin & Barrett, 2018). Consequently, early childhood serves as a key period to acquire social emotional and communicative developmental milestones (Soto-Icaza, Aboitiz & Billeke, 2015). Therefore, the current study aims to investigate the early appearance of ASD symptoms in young children with SCT.

Relevant to this developmental perspective is the notion that the presence of an additional X or Y chromosome is known to convergently impact the maturation of brain functions and networks involved in social adaptive cognitive and behavioral development, often referred to as the 'social brain' (Hong & Reiss, 2014; Raznahan et al., 2016). Atypical brain maturation may be expressed in an impaired development of social cognitive functions, necessary to shape social and communicative behavior in everyday life (Swaab, 2014). From this bottom-up neuropsychological perspective, having an extra X or Y chromosome may compromise social development and contribute to symptoms and clinical diagnoses of neurodevelopmental disorders such as ASD. Interestingly, since SCT can be identified as early as prenatally in contrast to ASD, early social developmental pathways can be studied prospectively. Identifying risk for ASD symptoms in genetic conditions such as SCT, may therefore give insights of etiological pathways leading to complex behavioral phenotypes, thereby serving as a naturalistic 'at risk' model of neurobehavioral development (Reiss, Eliez, Schmitt, Patwardhan & Haberecht, 2000). This investigation therefore explores the question how early social developmental pathways in young children with SCT may lead to social impairment later in life, as expressed in symptoms of ASD.

A pivotal dimension of infant social cognition that serves as an important milestone in early typical social development and as an early precursor for ASD is Joint Attention (Charman, 2003; (Dawson, Toth, Abbott, Osterling, Munson, Estes & Liaw, 2004; Franchini, Armstrong, Schaer & Smith, 2019). Joint Attention refers to the active capacity to coordinate attention between interactive social partners in order to share an awareness of an objects or event in their environment (Nation & Penny, 2008). In typical development, Joint Attention begins to emerge in the first 6 months of life and continues to develop at least until the age of 3 years old (Mundy & Newell, 2007). The early development of Joint Attention is manifest as two behavioral patterns: 1)

responding to Joint Attention, referring to the ability to follow the direction of gaze and gestures of others toward the object or event in their environment (also referred to as 'gaze following'), and 2) initiating Joint Attention, referring to the spontaneous use of direction of gaze and gestures to share or direct the attention of others toward the object or event, important for social understanding (Mundy, 2017). In typical early childhood, Joint Attention is an important social cognitive mechanism exposing the child to social experiences of perspective sharing with others. Observations of Joint Attention provide important information about the development of mental processes that are crucial for subsequent components of social and cognitive development that impact the understanding of and responding to social relevant information. For example, differences in Joint Attention abilities among young children are associated with language and global cognitive development (e.g. Delgado, Mundy, Crowson, Markus, Yale & Schwartz, 2002; Smith & Ulvund, 2003), and with social adaptation and self-regulation in preschool and school-aged children (e.g. Sheinkopf, Mundy, Claussen & Willoughby, 2004; Morales, Mundy, Crowson, Neal & Delgado, 2005). Accordingly, Joint Attention difficulties serve as a specific 'red flag' during social development of young children who are on developmental trajectories towards a clinical diagnosis of ASD, besides other pathologies like intellectual disabilities or specific language impairments (Rozga et al., 2011). Also in SCT, the development of Joint Attention abilities has proven to be vulnerable, as difficulties were found in the accuracy to spontaneously follow point and gaze gestures in children with SCT between 1 and 7 years old (Bouw, Swaab, Tartaglia & Van Rijn, 2021). Joint Attention can be reliably and objectively measured in behavioral observations and are feasible to implement as a marker for 'at-risk' subsequent developmental screening of social development (Nowell, Watson, Faldowski & Baranek, 2018). Because of the well supported developmental continuity between early Joint Attention and social (cognitive) and communicative development, the present study aims to explore whether Joint Attention abilities in young children with SCT longitudinally predict ASD symptoms in SCT.

Taken together, the primary aim of the current study is to investigate early pathways to social impairment as expressed in ASD symptoms in an international sample of young children with SCT, aged 1-8 years old. In addition to this main question, the predictive value of early Joint Attention on ASD symptoms one year later are explored in order to understand how early social cognitive mechanisms may predict

ASD symptoms in SCT. By identifying the effect of specific karyotype-subtype and possible recruitment bias, this study allows for an investigation of phenotypic differences within the SCT group. Based on the relevance of the extra X and Y chromosome on development of brain networks underlying social functioning and reported vulnerability for social adaptive functioning in individuals with SCT, we hypothesize that on average children with SCT may show increased levels of ASD symptoms, and that Joint Attention difficulties predict these symptoms over time.

Due to advances in noninvasive prenatal testing technology, the number of prenatal diagnoses of SCT is growing (Tartaglia et al, 2020). Given this rise in prenatal diagnoses of SCT, there is not only the opportunity to prospectively investigate early social development, but also the great need to gather knowledge on the early development of children with SCT. These studies will help to identify early targets for monitoring and early intervention, leading to improved clinical care and developmental outcomes for young children with SCT.

Methods

Recruitment

The present study is part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study - Leiden, The Netherlands), which includes children with SCT and nonclinical controls aged 1-8 years. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome.

Children with SCT were recruited and assessed at two sites: the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, 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 in cooperation with the clinical genetics and developmental pediatrics departments (from The Netherlands and Colorado, USA), as well as through patient-advocacy groups and social media postings. Recruitment strategy was assessed, and three subgroups were identified: (1) 'active prospective follow-up', which included families who were actively followed after prenatal diagnosis (52.4% 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 either after a prenatal or postnatal diagnosis (26.8% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development either after a prenatal or postnatal diagnosis (20.7% of the SCT group). All participants (child and parents) were Dutch or English speaking, and all children had normal or corrected-to-normal vision and did not have a history of traumatic brain injury or seizure disorder.

Participants

We obtained written informed consent from the parents/guardians of all participating children, according to the declaration of Helsinki. 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.

A group of 82 children with SCT (range 1-7 years old; M_{age}= 3.61, SD = 1.90) was included at baseline of the study. The study sample consisted of 24 girls with 47,XXX (29.2%), 41 boys with 47,XXY (50.0%) and 17 boys with 47,XYY (20.7%). The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping. Fifty-six children (68.3%) were diagnosed prenatally and 26 children were diagnosed postnatally (31.7%). Nineteen out of 41 boys with 47,XXY had received testosterone treatment (46.3%).

Parental education of caregivers was assessed at baseline, according to the criteria of Hollingshead (Hollingshead, 1975). Scores of this scale include: 0 (no formal education), 1 (less than seventh grade), 2 (junior high school), 3 (partial high school), 4 (high school graduate), 5 (partial college or specialized training), 6 (standard college/university graduation), and 7 (graduate/professional training). If two parents were available, level of education was averaged over both parents. Mean parental education was 5.95 (SD = 0.93). 95.6% of all parents indicated that their child has a second caregiver.

Measurements and instruments

Joint Attention. Joint Attention abilities were measured at baseline in a systematic behavior observation of a structured 20-min play situation, the Early Social

Communication Scales (ESCS; Mundy, 2003/2013). The ESCS is designed to measure the development of different dimensions of early nonverbal communication. Children were presented with a series of wind-up toys, hand-operated toys, and a book to look at with the experimenter that have been designed to elicit social and communicative bids with the experimenter. In addition, the experimenter presented the child with two sets of gaze-following trials. Data from the ESCS domains of Initiating Joint Attention and Responding Joint Attention were examined in this study. Following the procedures described by Mundy, (2003/2013) the ratings of Joint Attention during fixed time intervals were scored by trained independent raters. Coding consists of noting the frequency of occurrence of Joint Attention. Raters were not involved in the assessment, and blind to the child's karyotype. See Table 1 for a description of initiations of Joint Attention and responses to Joint Attention. The Joint Attention data for 1-2 years old children with SCT was already published in (Bouw, Swaab, Tartaglia & Van Rijn, 2021).

Table 1. Description of early joint attention behavior coded during social interactions (based on Mundy, 2003/2013).

Joint Attention		
Initiating Joint Attention	Eye contact	Child makes eye contact with examiner while manipulating or touching an inactive mechanical toy.
	Alternate	Child alternates looking at active mechanical toy or a toy in their hand and the examiner's eyes.
	Point	Child extends index finger toward toy within reach or to part of the room (e.g. posters).
	Show	Child extends toy toward the examiner's face.
Responding to Joint Attention	Look	Child turns head and eyes in the direction of the examiner's pointing gesture, or to the appropriate area of a book.

Symptoms of ASD in 2-4 years old children: M-CHAT. The Modified Checklist for Autism in Toddlers (M-CHAT) was administrated at follow-up to explore early signs of ASD in 2-4 years old children with SCT. The M-CHAT is an Autism Spectrum Disorder screener with 20 questions about current skills and behaviors, using a yes/no format (Robins, Casagrande, Barton, Chen, Dumont-Mathieu & Fein, 2014). Examples of items are: 'Does your child play pretend or make-believe?' and 'Does

your child respond when you call his or her name?' The Dutch version was translated from the original English version using a 'forward-back' translation approach by a bilingual native speaker of both languages (El-Behadli, Neger, Perrin & Sheldrick, 2015; Soto, Linas, Jacobstein, Biel, Migdal & Anthony, 2015). The M-CHAT was administrated to detect ASD symptoms in children with SCT aged 2-4 years old children. Presence of non-typical behavior was assigned a score of 1 and total score was interpreted. According to (Robins, Casagrande, Barton, Chen, Dumont-Mathieu & Fein, 2014), total score of 0-2 suggests a negative low-risk for ASD; total score of 3-7 as positive medium risk for ASD and total score of 8-20 as positive high risk for ASD. The M-CHAT has shown to have adequate sensitivity and specificity (Robins, Casagrande, Barton, Chen, Dumont-Mathieu & Fein, 2014).

Symptoms of ASD in 4-8 years old children: ADI-R. The Autism Development Interview - Revised (ADI-R) is a structured parent-report interview and recognized as the golden standard for establishing a clinical diagnosis of ASD (Rutter, Le Couteur & Lord, 2003; De Jonge, Greaves-Lord & De Bildt, 2014), and was administrated at follow-up to investigate ASD symptoms in 4-8 years old children with SCT. The ADI-R is based on DSM-IV and ICD-10 diagnostic criteria for ASD, includes over 100 items focused and generates algorithm scores for each of three subdomains of ASD symptomatology: (a) qualitative impairments in reciprocal social behavior (Social Interaction); (b) deficits in communication and language development (Communication) and (c) restricted range of interest and/or stereotypic behaviors (Restricted and Repetitive Behavior/Interests). The ADI-R interviews were scored by trained independent raters on the following scale: 0: "behavior of the type specified in the coding is not present"; 1: "behavior of the type specified is present in an abnormal form, but not sufficiently severe or frequent to meet the criteria for a 2"; 2: "definite abnormal behavior"; 3: "extreme severity of the specified behavior"; 7 for "definite abnormality in the general area of the coding, but not of the type specified"; 8 for "not applicable"; and 9 for "not known or not asked". To be consistent with DSM-5 diagnostic criteria for ASD (American Psychiatric Association, 2013), items from the Social and Communication domains were considered together. A sum of scores on the Social Interaction + Communication domain and on the Restricted and Repetitive Behaviors/Interests domain resulted in an ADI-R total score. We used the diagnostic algorithm, which is based on the (retrospective) functioning at age 4–5 year. For each primary domain of ASD a cut-off score is provided (Social

Interaction: cut-off score=10; Communication: cut-off score=8; Restricted and Repetitive Behaviors/Interests: cut-off score=3), above which a child meets clinical criterion for ASD.

Global level of cognitive development. In order to measure global level of intelligence at follow-up, developmental age-appropriate instruments were used. The Bayley Scales of Infant and Toddler Development, 3rd edition (cognitive subscale; Bayley-III, 2006) was administered to 2-4 years old children. In the older children four subtests of the Wechsler Preschool and Primary Scales of Intelligence, 3rd edition (WPPSI-III; Wechsler, 2002) were used to estimate global level of intelligence (Block Design, Matrix Reasoning, Vocabulary, and Similarities). The WPPSI-III was selected because there is a validated Dutch version. Total IQ estimates were calculated based on this short form version of the WPPSI-III (Hurks, Hendriksen, Dek & Kooij, 2016).

Study procedures

Assessment took place at various sites (Colorado (USA) and The Netherlands) 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 at each research setting. Researchers from Leiden University were responsible for all project and data-management (i.e., training and supervision of researchers, processing and scoring of data). All assessments and questionnaires in the study were administrated by child psychologists in the Dutch or English language, depending on the first language of the child and parents. During the ESCS, the child was seated at a table across from a familiar examiner (see Figure 1 for the set-up of the assessment room), and verbal interactions were kept to a minimum. The structured ESCS assessment was videotaped, with full face view of the child and profile view of the experimenter. At a follow-up assessment, one year (=12 months) after baseline, the Bayley-3, WPPSI-III, M-CHAT and ADI-R were administered. The M-CHAT questionnaire was filled in by the primary caregiver of children aged 2-4 years, and the ADI-R interview was conducted with the primary caregiver of children with SCT, aged 4-8 years old children.

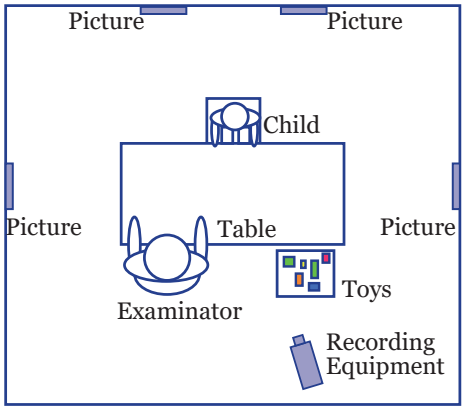

Figure 1. Set-up assessment room ESCS administration (adapted from: Mundy, 2003/2013).

Statistical analyses

Statistical Package for the Social Sciences (SPSS, version 25) was used for statistical analyses. An independent sample t-test was used to measure research site differences (The Netherlands vs. USA) on the outcome variables in the SCT group, and differences in ASD symptoms between two IQ groups (below average; average IQ). Pearson's *r* was used to assess correlations between age, global cognitive functioning, ASD symptoms and Joint Attention. Hierarchical linear regression models were carried out to investigate the longitudinal relationships between Joint Attention and ASD symptoms, accounting for age effects (first step: age of the child; second step: Joint Attention). Differences on outcome measures between karyotype-subtypes and recruitment groups were measured with ANCOVAs, accounting for age effects. Statistical analyses were performed two-tailed, and statistical significance was set at $p < .05$ a priori. Effect sizes were calculated with R^2 (explained variance).

Results

Comparison between research sites

First, to control for the potential impact of research site (and therefore for cultural bias/response tendencies) on outcomes of the study, the data of the two research sites were compared. No differences between research sites (The Netherlands, USA) were found for total scores on the primary measure of ASD symptoms, including the M-CHAT (t (26) = -1.20, p = .241), and for ADI-R total score (t (53) = -1.53, p = .132).

Therefore, data were collapsed across sites.

Joint Attention at baseline

The ESCS was successfully completed by 79 children (three children were not able to complete the task). Interrater reliability was measured based on a subsample of ten participants and show an intraclass correlation coefficient (ICC) of 0.81-0.99 (for the Joint Attention scales collapsed together) which is considered to reflect excellent reliability (Cicchetti & Sparrow, 1981). Joint Attention on the baseline ESCS assessment was significantly correlated with age (p <.001). See Table 2 for descriptive statistics for Joint Attention in two SCT age groups (1-3 years old; 3-7 years old).

Table 2. Descriptive statistics of joint attention behaviors (initiating and responding) in children with Sex Chromosome Trisomies.

	1-3 years old *n* = 27		**3-7 years old** *n* = 52		**Correlations between JA and age**
Joint Attention (ESCS: raw score)	min-max	*M (SD)*	min-max	*M (SD)*	*Pearson's r, p-value*
Initiating Joint Attention	2-43	18.07 (10.37)	0-89	30.00 (17.43)	r =.423, p <.001
Responding to Joint Attention (max. score = 12)	1-10	6.59 (3.08)	3-12	9.46 (1.58)	r =.523, p <.001

Note: JA = Joint Attention; ESCS = Early Social Communication Scales.

Clinical risk for ASD at follow-up

The study group was divided into low risk and high risk based on the scoring algorithms of the M-CHAT and ADI-R as described above. In order to investigate overall clinical risk for the diagnosis of ASD in children with SCT across the 2-8 year age span, the M-CHAT and ADI-R data were collapsed. Out of 82 children with SCT, 18 children (22.0%) were classified as at clinical risk for ASD.

ASD symptoms at different ages

To investigate symptoms of ASD at different developmental ages, the sample was divided into two age groups: children aged 2-4 years old (N = 28; 4 XXX, 20 XXY, 4 XYY), and children aged 4-8 years old (N = 54; 20 XXX, 21 XXY, 13 XYY). The distribution of karyotypes (XXX, XXY, XYY) was different in the two age groups (χ^2 (2) = 0.02, p = .018), due to the high number of XXY-participants in the younger age group.

2-4 years old children with SCT. ASD symptoms were assessed in the 2-4 years olds (N=28) using the M-CHAT. Within the SCT group, the mean score is 2.35 (SD = 2.06). Based on the manual of the M-CHAT, out of 28 children, 5 children were classified as moderate/high risk for ASD (17.9%), and 23 children (82.1%) were classified as low risk. Total score on the M-CHAT was not correlated with age (r = .137, p = .488).

4-8 years old children with SCT. The ADI-R interview was used to assess early ASD symptoms in the 4-8 year age group (N = 54). Within the SCT group, mean scores in the Social Interaction + Communication domain was 20.22 (SD = 12.46), and in the Restricted Interests and Repetitive Behavior domain 2.57 (SD = 2.99). Within the SCT group, out of 54 children, 13 children (24.1%) scored above cut-off on all domains. For an overview of the percentages of children scoring above cut-off on the domains of 'Social Interactions + Communication', 'Restricted Interests and Repetitive Behavior', and on all domains, see Table 3. Total scores on the ADI-R were not correlated with age at follow-up (r = .209, p = .129).

Table 3. Percentage of children with SCT with ADI-R scores above cutoff.

	% children with SCT
Above cut-off on all domains	24.1%
Above cut-off on 'Social Interactions' + 'Communication'	72.2%
Above cut-off on 'Restricted Interests and Repetitive Behavior'	25.9%
Below cut-off on all domains	7.3%

Note: SCT = Sex Chromosome Trisomy.

Role of global cognitive functioning

Within the SCT group, ASD symptoms were not correlated with global cognitive functioning (M-CHAT total score and Bayley cognitive composite score: r = -.016, p =.938; ADI-R total score and WPPSI total IQ: r = -.298, p =.052). Because of the borderline p-value of the negative correlation between ADI-R total scores and WPSSI total IQ (p = .052), we investigated whether ASD symptoms are more pronounced in children with SCT with a below average IQ. Total IQ was categorized into two groups (IQ < 84: below average; IQ >85: average). The distribution of karyotypes (XXX, XXY, XYY) was similar between the two IQ groups (χ^2 (2) = .37, p = .831). An independent t-test was carried out to investigate differences in ASD symptoms between children with SCT in both IQ-groups. No significant differences were found between children with SCT in the below average IQ group (M = 25.78, SD = 16.83), and the average IQ group (M = 21.25, SD = 14.70; t (43)= 0.81, p = .426). These results indicate that on average, SCT children with below average and average IQ have comparable amounts of ASD symptoms, although the correlation between lower cognitive scores and ASD symptoms in the older age group should be acknowledged.

Predictive value of Joint Attention on ASD symptoms

In order to investigate whether initiating Joint Attention and responding to Joint Attention at baseline are predictors for early ASD symptoms at follow-up, hierarchical regression analyses were carried out in the two separate age groups, accounting for age effects.

2-4 years old children. No significant predictive relationship was found between initiating Joint Attention and ASD symptoms in children with SCT, aged 2-4 years (F (2,24) = 0.24, p = .789). However, responding to Joint Attention did significantly predict ASD symptoms in 2-4 years old children with SCT (F (2,24) = 4.24, p = .049; b = -.26, β = -.38, R^2 = .15), indicating that a lower frequency of responses to Joint Attention significantly predict more ASD symptoms.

4-8 years old children. In 4-8 years old children with SCT, a significant predictive relationships was found between initiating Joint Attention and ASD symptoms (F (2,49) = 4.92, p = .011; b = -.30, β = -.36, R^2 = .17). Similarly, a significant predictive relationship was found between responding to Joint Attention and ASD symptoms

(F (2,49) = 3.23, p = .048; b = -2.44, β = -.26, R^2 = .12). These results indicate that lower frequencies of initiations of Joint Attention and responses to Joint Attention significantly predict more ASD symptoms in 4-8 years old children with SCT. See Figure 2 for an overview of the significant relations between Joint Attention and ASD symptoms in the two age groups.

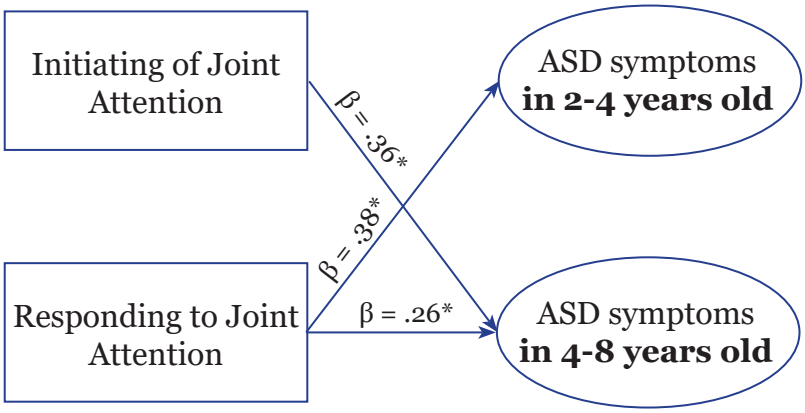

Figure 2. Significant longitudinal predictive relationships between Joint Attention and ASD symptoms in 2-4 years old children and 4-8 years old children with Sex Chromosome Trisomies.

Note: * = p <.05.

Karyotype differences

As the distribution of karyotypes was different between the two age groups, ANCOVAs were carried out to measure differences between the various karyotypes on ASD symptoms in children aged 2-4 year (M-CHAT), and in children aged 4-8 year (ADI-R), accounting for age effects. For ASD symptoms in 2-4 years old children with SCT, no differences between karyotypes were found (F (1,24) = 0.53, p = .571). Similarly, for ASD symptoms in 4-8 years old children with SCT, no differences between karyotypes were found (F (1,51) = 0.88, p = .419), indicating a similar impact of the extra X or Y chromosome on ASD symptoms. See Table 4 for exact M, SDs and p-values.

Table 4. Differences in ASD symptoms across karyotypes (M, SD).

ASD symptoms	XXX	XXY	XYY	*p*-value
2-4 years old children	*n* = 4	*n* = 20	*n* = 4	
M-CHAT: Total score	2.50 (1.73)	2.55 (2.28)	1.25 (0.50)	.571
4-8 years old children	*n* = 20	*n* = 21	*n* = 14	
ADI-R: Total score	22.85 (13.41)	19.90 (12.78)	26.86 (17.26)	.419

Note: ASD = Autism Spectrum Disorder.

Recruitment bias

Within the SCT group we used ANOVAs to test for differences on total scores between the three recruitment groups (prospective follow-up after prenatal diagnosis/ information seeking parents/clinically referred cases), accounting for age effects. There were no differences in recruitment groups on ASD symptoms in 2-4 years old children with SCT (F (1,24) = 0.24, p = .786) or in 4-8 years old children with SCT (F (1,51) = .53, p = .571). These results show that how children with SCT enrolled in the study is not related to their level of ASD symptoms. See Table 5 for exact *M*, *SD*s and *p*-values.

Table 5. Differences in ASD symptoms across recruitment strategies (M, SD).

ASD symptoms	Prospective follow-up	Information seeking parents	Clinically referred cases	*p*-value
2-4 years old children	*n = 17*	*n = 9*	*n = 2*	
M-CHAT: Total score	2.59 (2.48)	2.11 (1.27)	1.50 (0.71)	.786
4-8 years old children	*n = 27*	*n = 13*	*n = 15*	
ADI-R: Total score	20.67 (14.67)	24.77 (13.80)	24.73 (14.28)	.523

Note: ASD = Autism Spectrum Disorder ; SCT = Sex Chromsome Trisomie.

Discussion

The current study aims to investigate the prospective impact of Sex Chromosome Trisomies (SCTs; XXX, XXY, XYY) on the appearance of early symptoms of Autism Spectrum Disorder (ASD), in children aged 1-8 years old, with specific emphasis

on the predictive value of Joint Attention on these ASD symptoms. The biological predisposition of SCTs allows us to study early social developmental pathways of a homogeneous group of children, and may be considered an 'at risk' group when it comes to neurobehavioral social adaptive development and related psychopathology (Van Rijn, 2019; Reiss, Eliez, Schmitt, Patwardhan & Haberecht, 2000), since the risk for ASD in this group has been found to be about 15-30% in children and adolescents (Van Rijn, 2019). The results of our study add to the understanding of the early prospective contribution of the extra X or Y chromosome on clinical risk for behavioral defined neurodevelopmental disorder such as ASD, and of early Joint Attention mechanisms predicting these ASD symptoms. The results demonstrate that ASD symptoms are substantially higher in children with SCT between 2 and 8 years old as compared to the general population, with 22% of the children at clinical risk for ASD, based on parental reports.

The most important conclusion of our study is that clinical diagnostic levels of ASD symptoms in SCT may already be seen in 22% of very young children. Several studies examining risk for ASD symptoms at later ages in childhood and adolescence with various types of outcome measures and different SCT populations have found similarly elevated symptoms of ASD and related increased clinical diagnoses of ASD in individuals with SCT from school-age into adolescence (Cederlöf et al., 2014; Ross et al., 2012; Tartaglia et al., 2017; Wigby et al., 2016; see for a review: Van Rijn, 2019). The results of the current study add to the literature by showing that a neurodevelopmental impact of the extra X or Y chromosome is already detectable in very young children with SCT that show ASD symptoms. ASD symptoms are similarly reported across all three karyotypes (XXX, XXY, XYY). Ascertainment bias and site of recruitment were not found to be relevant to the percentages of ASD symptoms, indicating the robustness of these results.

By comparing two developmental age groups (2-4 years and 4-8 years) we were able to assess ASD symptoms during different developmental phases of early childhood. We found a relatively comparable impact of SCT on the appearance of early ASD symptoms at both age ranges (respectively, 18% and 24%). These ASD symptoms are not associated with global cognitive functioning scores, indicating that, on average, young children with SCT show an equal amount of ASD symptoms across different levels of cognitive functioning, although further examination of this relationship at

older aged when cognitive assessments become more predictive of later functioning will be important.

By using the ADI-R interview, we assessed a profile of ASD symptoms in children from the age of 4 years and older. Although 24% of the children scores above cut-off on all domains of the ADI-R (Social Interaction + Communication, and Restricted Interests and Repetitive Behavior), we found that in 72% of the children scores on the Social Interaction+Communication domain reached clinical diagnostic thresholds. These results indicate a specific profile of ASD symptoms in young children with SCT, with relatively more vulnerabilities on the social and communicative domain, as compared to the whole conglomerate of ASD symptoms including restricted interests and repetitive behaviors.

Considering the impact of social impairments on interpersonal interaction and the development and maintenance of satisfying relationships with others (Rao, Beidel & Murray, 2008), these early ASD symptoms may indicate an 'at risk' social pathway of a considerable percentage of children with SCT. However, it is important to note that there is also a subgroup of children with SCT that show no ASD symptoms in the clinical range. Thus, while a considerable percentage of parents express their concerns about the social development of their young child with SCT, there is also a group of young children with SCT with no or only some symptoms of ASD. Further, it is important to emphasize that the presence of some ASD symptoms does not mean that the child meets full clinical criteria for a diagnosis of ASD.

The second aim of this study was to explore the predictive value of Joint Attention, as an early social cognitive mechanism precursing ASD symptoms in young children with SCT. We used systematic behavior observations of Joint Attention during a structured play situation to evaluate the degree to which responding to another's joint attention bids (i.e. Responding to Joint Attention) and the tendency to initiate joint attention episodes (i.e. Initiations of Joint Attention) are related to ASD symptoms. Our results demonstrate that in young children with SCT, the tendency to coordinate attention between social partners (i.e. Joint Attention) is predictive of ASD symptoms at one year follow-up. In SCT, the early development of Joint Attention abilities has proven to an area of vulnerability (Bouw, Swaab, Tartaglia & Van Rijn, 2021), and the current results have implications for our understanding

of the likely consequences of vulnerabilities in the capacity to form Joint Attention in young children with SCT. Low tendencies of following or initiating non-verbal communicative cues early in development may contribute to longer term impairments in adaptive social communication. Specifically, from earlier studies it is known that Joint Attention is essential in social-cognitive development underlying social adaptive behavioral development; and the practice of Joint Attention provides experiences of perspective sharing for the child (Mundy & Neal, 2000; Mundy & Newell, 2007). A lower tendency to engage in Joint Attention therefore may lead to a cascade of negative developmental effects, not only in core social cognitive skills, but also in language development and social adaptive behavioral functioning (Nyström, Thorup, Bölte & Falck-Ytter, 2019); and indeed we found that in SCT, Joint Attention mechanisms serve as early predictors of pathways for risk of severe social behavioral impairments, as indicated by symptoms of ASD.

Interestingly, we found differences in the two age groups with regard to the specific type of Joint Attention in predicting ASD symptoms at a specific age range: whereas only *responding* to Joint Attention predicts ASD symptoms in 2-4 years old children with SCT, both *responding* to and *initiating* Joint Attention predicted ASD symptoms in children aged 4 years and older. This finding supports the hypothesis that responding to Joint Attention and initiating Joint Attention have separate developmental trajectories and contribute differently to subsequent developmental outcomes (Mundy & Jarrold, 2010; Kim & Mundy, 2012). Reduced responses to Joint Attention (i.e. RJA) are often observed during early childhood in children at risk for ASD. The initiation of Joint Attention seems to be even more critical and persistent in severe social impairments from early childhood into school age and adolescence (Nyström, Thorup, Bölte & Falck-Ytter, 2019). Neuroimaging studies show that brain networks supporting responding to Joint Attention and *initiating* Joint Attention are partially distinctive (see for a review: Mundy, 2018). Specifically, *initiating* Joint Attention is associated with increased activation of brain areas associated with reward processing (Schilbach et al., 2010; Gordon, Eilbott, Feldman, Pelphrey & Van der Wyk, 2013), suggesting that initiation of Joint Attention may be more related to social motivation, as compared to responding to Joint Attention that may be triggered more automatically and spontaneously. In conclusion, the results of the second part of our study indicate that, as children with SCT develop, challenges with the complex ability to initiate Joint Attention in which social motivation is

believed to be involved, may be a pivotal precursor in the emergence of severe social impairments and the related risk for ASD symptoms; this, combined with the ability to spontaneously follow Joint Attention bids from social partners is important across both age groups in this investigation.

The outcomes of our study could also provide insight in sex differences that are observed in neurodevelopmental disorders. ASD has been found to be more prevalent in males as compared to females and has different clinical manifestations in males and females (Riecher-Rössler, 2017; Mandy, Chilvers, Chowdhury, Salter, Seigal, & Skuse, 2012). The understanding of these sex differences in ASD have important implications for tailored and individualized clinical care by guiding diagnosis and (preventive) intervention. The study of individuals with SCT provides a natural framework for the study of sex differences in neurodevelopmental disorders, as they provide us by a model for examining the effects of alterations in sex chromosome number on risk of ASD (Green, Flash, & Reiss, 2019). By comparing SCT conditions (XXX vs. XXY vs. XYY) we found no differences between karyotypes, indicating a similar impact of the extra X or Y chromosome on clinical risk for ASD between the age of 2-8 years. However, these findings are not in line with earlier studies that found increased ASD symptoms in school-aged boys and adolescents with XYY as compared to boys with XXY and typical controls (see for example: Ross et al., 2012), and with increased ASD symptoms in 3-7 year old boys with XYY as compared to boys and girls with an extra X chromosome and typical controls (Bouw, Swaab, Tartaglia, Cordeiro, Van Rijn, 2022). Together, these data suggest that the Y chromosome in and of itself may be correlated with an increase in ASD susceptibility that become more pronounced in older age groups. However, it should be considered that the distribution of karyotypes (XXX, XXY, XYY) was unequal across age groups (2-4 years; 4-8 years). Although no effect of karyotype-subtype was found on ASD symptoms in both age groups in the current study, the specificity of early developmental pathways of karyotype-subtypes remain unclear, which calls for further studies with larger sample sizes. Direct comparisons between different SCT conditions in addition to comparison with age related peers without an extra X or Y chromosome (XX, XY) would provide important evidence about the role of sex chromosomes on neurodevelopmental outcomes.

Overall, the results of the present study have important implications for clinical care.

As ASD symptoms were found at the early age of 2 years, these findings underscore the importance of incorporating closely monitoring social adaptive functioning and screening for ASD symptoms in children with SCT as routine care within the first years of life. Because of the predictive relationship between Joint Attention and ASD symptoms as found in this study, difficulties with Joint Attention can serve as an early marker for an 'at risk' developmental profile and is a potential target for early intervention. Early detection and support of children 'at risk' is relevant for three reasons. First, children at risk for social impairments may have fewer social learning experiences compared to their peers, possibly leading to a cascade of negative developmental effects (Mundy & Newell, 2007). Second, parents of children with known SCT often experience stress and uncertainty about their child's development and their own parenting (Richardson, Riggan & Allyse, 2021). Early detection of developmental risk in children allows for appropriate support for parents by offering psychoeducation or coaching. Early support of parents might result in higher parent well-being and family adaptive functioning by means of improved positive psychological functioning and allow for learning effective coping strategies (see for examples about the efficacy of early parental support in the ASD literature: Estes, Swain & MacDuffie, 2019; Saccà, Cavallini & Cavallini, 2019). Finally, preventive support and treatment early in life of children with SCT might reduce vulnerability for social impairments later in life. It is therefore of high relevance to evaluate the efficacy of neurocognitive and neurobehavioral interventions early in life of children with SCT. As early interventions are well established to support the early development of children with ASD and their families (see for a review: French & Kennedy, 2018), these early services may be beneficial for young children with SCT as well (Tartaglia et al., 2017). Unfortunately, research evaluating the potential effects of early intervention and parental support in the SCT population remains scant, although there is recent promising evidence that early neurocognitive training can support social cognitive development of 4-8 years old children with SCT (Bouw, Swaab & Van Rijn, 2022). It is of urgent need to further investigate the possible efficacy and long-term outcomes of services and programs early in life of children with SCT, even more considering the need to offer supportive preventive interventions to the growing group of (prenatally) diagnosed children with SCT (Samango-Sprouse, Keen, Sadeghin & Gropman, 2017; Tartaglia et al., 2020).

The present study has several limitations, thus providing suggestions for future

studies. First, our study on the early impact of SCT on ASD symptoms relies on parental reports which might represent bias. Behavioral symptoms of the child were not observed or discussed in a multidisciplinary team of clinicians to establish a shared clinical perspective. However, former studies demonstrated that parental assessment of ASD symptoms based on the M-CHAT and ADI-R correlates with behavioral observations of ASD symptomatology during clinical observations, for example during the Autism Diagnostic Observation Scale (ADOS; Robins, Casagrande, Barton, Chen, Dumont-Mathieu & Fein, 2014; Lord, Rutter & Le Couteur, 1994). Second, although a percentage of boys with XXY (Klinefelter Syndrome) received testosterone treatment (46%), the sample is not powered to study the efficacy of testosterone treatment on ASD symptoms. Randomized and placebo-controlled trials would provide more reliable insights into the effects of testosterone treatment in boys with Klinefelter syndrome (which is recently designed and running: PI Davis, NCT03325647). Finally, longer prospective follow-up of the cohort beyond one year is important to determine if these predictive relationships remain relevant as more complex social and communications skill develop, and whether Joint Attention and/or other factors are indeed predictive for a later clinical diagnosis of ASD.

To conclude, the current longitudinal study with a relatively large and international sample of young children with SCT demonstrates the appearance of early ASD symptoms in a subset of young children with SCT, from 2 years of age onward. Moreover, we found that Joint Attention, and especially the capacity to initiate Joint Attention, longitudinally predicts these ASD symptoms. These results advocate for close monitoring and early (preventive) support and intervention, aiming to optimize social adaptive development in young children with SCT.

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CHAPTER 7

Early preventive intervention for young children with Sex Chromosome Trisomies (XXX, XXY, XYY): Supporting social cognitive development using a neurocognitive training program targeting facial emotion understanding

Bouw, N., Swaab, H., & Van Rijn, S. (2022). Early preventive intervention for young children with sex chromosome trisomies (XXX, XXY, XYY): supporting social cognitive development using a neurocognitive training program targeting facial emotion understanding. *Frontiers in psychiatry, 13*.

Abstract

Objective: Sex Chromosome Trisomies (SCT; XXX, XXY, XYY) are genetic conditions that are associated with increased risk for neurodevelopmental problems and psychopathology. There is a great need for early preventive intervention programs to optimize outcome, especially considering the increase in prenatal diagnoses due to recent advances in noninvasive prenatal screening. This study is the first to evaluate efficacy of a neurocognitive training in children with SCT. As social behavioral problems have been identified as among the key areas of vulnerability, it was targeted at improving a core aspect of social cognition, the understanding of social cues from facial expressions.

Methods: Participants were 24 children with SCT and 18 typically developing children, aged 4-8 years old. Children with SCT were assigned to a training (n = 13) or waiting list (no-training) group (n = 11). Children in the training group completed a neurocognitive training program (The Transporters), aimed to increase understanding of facial emotions. Participants were tested before and after the training on facial emotion recognition and Theory of Mind abilities (NEPSY-II), and on social orienting (eyetracking paradigm). The SCT no-training group and typically developing control group were also assessed twice with the same time interval without any training. Feasibility of the training was evaluated with the Social Validity Questionnaire filled out by the parents and by children's ratings on a Visual Analogue Scale.

Results: The SCT training group improved significantly more than the SCT no-training and TD no-training group on facial emotion recognition (large effect size; η_p^2 =.28), performing comparable to typical controls after completing the training program. There were no training effects on ToM abilities and social orienting. Both children and parents expressed satisfaction with the feasibility of the training.

Conclusions: The significant improvement in facial emotion recognition, with large effect sizes, suggests that there are opportunities for positively supporting the development of social cognition in children with an extra X or Y chromosome, already at a very young age. This evidence based support is of great importance given the need for preventive and early training programs in children with SCT, aimed to minimize neurodevelopmental impact.

Introduction

Between 1:650 to 1:1000 children are born with a Sex Chromosome Trisomy (SCT; Boyd, Loane, Garne, Khoshnood, & Dolk, 2011). SCT are characterized by an extra X or Y chromosome compared to the typical karyotype of 46,XX in girls and 46,XY in boys. Intellectual functioning is typically within normal limits, although somewhat lower on average, and SCT is related with a profile of specific cognitive vulnerabilities, for example in areas of executive functioning, language and social cognition (see for reviews: Leggett, Jacobs, Nation, Scerif, & Bishop, 2010; Urbanus, Van Rijn, & Swaab, 2020). Children and adolescents with SCT also show higher percentages of clinical diagnoses of neurodevelopmental disorders, such as Attention Deficit/ Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD; Cederlöf et al., 2014; Ross et al., 2012; Van Rijn, 2019).

As SCT are conditions that are associated with increased risk for neurocognitive vulnerabilities and related neurobehavioral problems, these genetic conditions may serve as naturalistic 'at risk' models of neurodevelopment. More specifically, the presence of an additional X or Y chromosome is known to convergently impact the maturation of brain functions and networks involved in social adaptive cognitive and behavioral development, often referred to as the 'social brain' (Hong & Reiss, 2014; Raznahan et al., 2016). Therefore, the use of specific genetic conditions as models of more common behavioral and cognitive developmental disorders can reveal insights into early neurodevelopmental pathways that contribute to neurodevelopmental and -behavioral dysfunction in children. Therefore, research of the impact of genetic conditions such as SCT on development and potential effective interventions supporting development will help to elucidate the linkages among genetic, neurocognitive and neurobehavioral development.

Due to recent advances in noninvasive prenatal testing technology (i.e. the introduction of the NIPT; Samango-Sprouse, Keen, Sadeghin, & Gropman, 2017; Tartaglia et al., 2020), it is possible to identify SCT as early as prenatally, resulting in increasing diagnoses of SCT. Give this rise in prenatal diagnoses of SCT, there is not only the opportunity to prospectively investigate early development, but also the opportunity and urgent need to study whether early preventive interventions may possibly reduce risk for difficulties in adaptive functioning and psychopathology later in life (Herlihy & McLachlan, 2015). However, to date, there has been no research

evaluating the potential effects of early and preventive neurocognitive training in SCT. The present study aims at providing in this.

In defining the targets for early intervention in SCT, a key area may present the social domain, considering that social adaptation is among the key domains of vulnerability in SCT (Van Rijn, 2019; Urbanus, Van Rijn, & Swaab, 2020; Tartaglia et al., 2020). Underlying cognitive mechanisms that may drive the risk of these social behavioral difficulties are social cognitive functions, referring to the mental processes that are used to perceive and process social cues, stimuli and environments, and underpin social adaptive functioning (Beauchamp & Anderson, 2010). With respect to SCT, recent reviews identify social cognition as among the key areas of difficulty from school age on (Urbanus, van Rijn, & Swaab, 2020; van Rijn, 2019). Although outcomes are variable, reported vulnerable social cognitive abilities include reading social signals from social gaze directions, facial emotion understanding, face processing (accuracy and reaction time) and Theory of Mind, referring to the attribution of mental states, intentions and emotions to others (Frith & Frith, 2003). Calculated effect sizes indicated high to very high clinical significance. Interestingly, specific age dynamics during early development of social cognitive functions in young children with SCT were recently found (Bouw, Swaab, Tartaglia, & Van Rijn, 2021), often described as the 'growing into deficit' phenomenon (Rourke et al., 1983), the effect that development is increasingly deviating compared to typical developing peers when children become older. Early intervening with children who are 'at risk' for adverse development, but do not yet exhibit full expression of the syndrome, may provide the best advantages from intervention. By implementing intervention early in life, the course of social development may be shaped, preventing for adverse long-term outcomes (Dawson, 2008). Given the difficulties in underlying social cognitive mechanisms in SCT that serve as building blocks for social adaptive functioning (Beauchamp & Anderson, 2010), it is important to study whether it is possible to support the development of social neurocognitive functions early in development by early intervention trials.

Training of emotion perception and understanding appears to be an important component of effective social cognitive interventions (Roelofs, Wingbermühle, Egger, & Kessels, 2017). In addition, emotion recognition develops already early in life (Soto-Icaza, Aboitiz, & Billeke, 2015), is proven to be vulnerable across the life-span

of individuals with SCT, and therefore an important target to preventively support social cognitive development early in life of individuals with SCT. In typical early social cognitive development, the ability to recognize facial expressions correctly and to respond to them appropriately is vital for successful everyday social interaction, and a prerequisite for showing social adaptive behavior, responsive of social feedback that follows social interactions. This ability to recognize facial emotions, in turn, depends on basic social orientation, the spontaneous visual orienting of attention to naturally occurring and meaningful aspects of social interactions (i.e. eyes and faces), which is already present in the first 6 weeks of postnatal life (Johnson, 2005b); for a review on eye tracking studies, see Reynolds & Roth, 2018). Later developing and higher order social cognitive skills as for example Theory of Mind likely depend on this very early propensity to orient attention to social important information and to recognize facial emotions (Rochat & Striano, 1999). Attributing and understanding mental states such as beliefs, desires and intentions of others and oneself with the ability to share these during social interactions (i.e. Theory of Mind) continue to develop throughout childhood, based on maturation of complex neural networks and high-order cognitive processes (Happé & Frith, 2014). We evaluated the efficacy of an emotion recognition training program in SCT on key areas of typically social cognitive development during early childhood that were found to be vulnerable in young children with SCT, i.e. on measures of emotion recognition, Theory of Mind and social orientation (Bouw, Swaab, Tartaglia, & Van Rijn, 2021).

Most of the currently available training programs targeted to enhance emotion recognition are computer-based. These computer-based neurocognitive training programs provide the opportunity to teach emotion recognition in a controlled and structured environment with little social demands. Motivation and interest are usually easily to maintain, and materials are low-budget and therefore easy accessible (Moore, McGrath, & Thorpe, 2000; Berggren, Fletcher-Watson, Milenkovic, Marschik, Bölte, & Jonsson, 2018).

The efficacy of a home-based computer-based emotion recognition training in young children with SCT in the current study was evaluated by comparison of measures of emotion recognition, Theory of Mind and social orienting before and after the training. Training effects in young children with SCT were compared with two groups that did not attend the training program i.e. a waiting list group with SCT, and a group

of typically developing children. Also, we studied feasibility and implementation of the training program in the SCT training group, based on self-report of the parents, and the children. Because studies on the efficacy of neurocognitive training that target to support early social cognitive development in SCT are lacking, this study is unique and may provide important implications for clinical care and future research aimed at improving evidence-based care for children with SCT in order to support optimal neurodevelopmental outcome.

Materials and methods

Recruitment

The present study is part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study – Leiden, The Netherlands), which includes children with SCT and nonclinical controls. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. Recruitment and assessment of the current study took place as part of this larger study, at the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, The Netherlands. Children in the SCT group were recruited in cooperation with clinical genetics departments in the Dutch speaking parts of Western Europe.

Typically developing control children were recruited from the western part of The Netherlands, and approached with information brochures about the study. All participants were Dutch speaking, had normal or corrected-to-normal vision, and did not have a history of traumatic brain injury. The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping. For ethical reasons, children in the typically developing group were not subjected to genetic screening, As the prevalence of SCT is ~1 in 1000, the risk of having one or more children with SCT in the typically developing group was considered minimal and acceptable.

Participants

A group of 25 children with SCT (range 4-8 years old) was included in this study. Children with SCT were assigned to a training group (SCT training group; n =14) or waiting list no-training group (SCT no-training group; n =11). See Table 1 for

background information of the participants. Likelihood Ratio tests were performed to investigate the ratios of age, gender and karyotype distribution across the study groups. Mean age across the three study groups did not differ between the three groups (F (2,39) = 0.58, p = .566). Also, gender distribution did not differ between the three study groups (λ(2) = 2.96, p =.227). There was no difference in distribution of karyotypes between the SCT training and SCT no-training group (λ (2) = 3.50, p =.187). One girl with 47,XXX in the SCT training group dropped out of the study during the second week, as she was not motivated to continue watching the training episodes any longer.

For the SCT group, recruitment strategy was assessed, and three subgroups were identified: (1) 'active prospective follow-up', which included families who were actively followed after prenatal diagnosis (58.3% 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 (20.8% of the SCT group), and (3) 'Clinically referred cases', which included families seeking professional help based on specific concerns about their child's development (20.8% of the SCT group). The distribution of recruitment strategy did not differ between the SCT training and SCT no-training group (λ(2) = 2.25, p =.325). One out of nine boys with 47,XXY had received testosterone treatment (11%). Testosterone treatment was performed at the age of 1 year, 3 years before the start of the current intervention study.

Parental education of the primary caregiver was assessed, according to the criteria of Hollingshead (Hollingshead, 1975). Scores of this scale include: 0 (no formal education), 1 (less than seventh grade), 2 (junior high school), 3 (partial high school), 4 (high school graduate), 5 (partial college or specialized training), 6 (standard college/university graduation), and 7 (graduate/professional training). 81% of all parents indicated that their child has a second caregiver. If two parents were available, level of education was averaged over both parents. No differences in parental education distribution between the three study groups were found (λ(16) = 15.36, p = .498).

Table 1. Background information of participant.

	SCT training group	SCT no-training group	TD control group
Age	5.86 (1.28)	6.33 (1.27)	5.87 (1.15)
Gender	5 boys, 8 girls	8 boys, 3 girls	9 boys, 9 girls
Parental education	5.58 (1.08)	6.10 (0.83)	5.58 (1.41)
Karyotype	8 XXX, 4 XXY, 1 XYY	3 XXX, 5 XXY, 3 XYY	n.a.
Recruitment strategy*	A:6, B:4, C:3	A:8, B:1, C:2	n.a.

Note: T = Sex Chromosome Trisomy; TD = Typically Developing.
* A = Prospective follow-up, B = Information seeking parents, C = Clinically referred cases.

Design of the study

The current study had a repeated measures within-subject design. All participants were assessed twice: at baseline and 4 weeks later (follow-up). At baseline global level of cognitive functioning was measured, as well as receptive and expressive language skills. At both baseline and follow-up children facial emotion recognition, Theory of Mind, and social orienting was assessed. Between the baseline and follow-up assessment, children in the SCT training group participated in the emotion training program. Children in the SCT no-training group and the typical control group completed the baseline and follow-up assessment, but did not participate in the training program between baseline and follow-up, nor in other forms of early intervention as part of regular care. See Figure 1 for an overview of the study design.

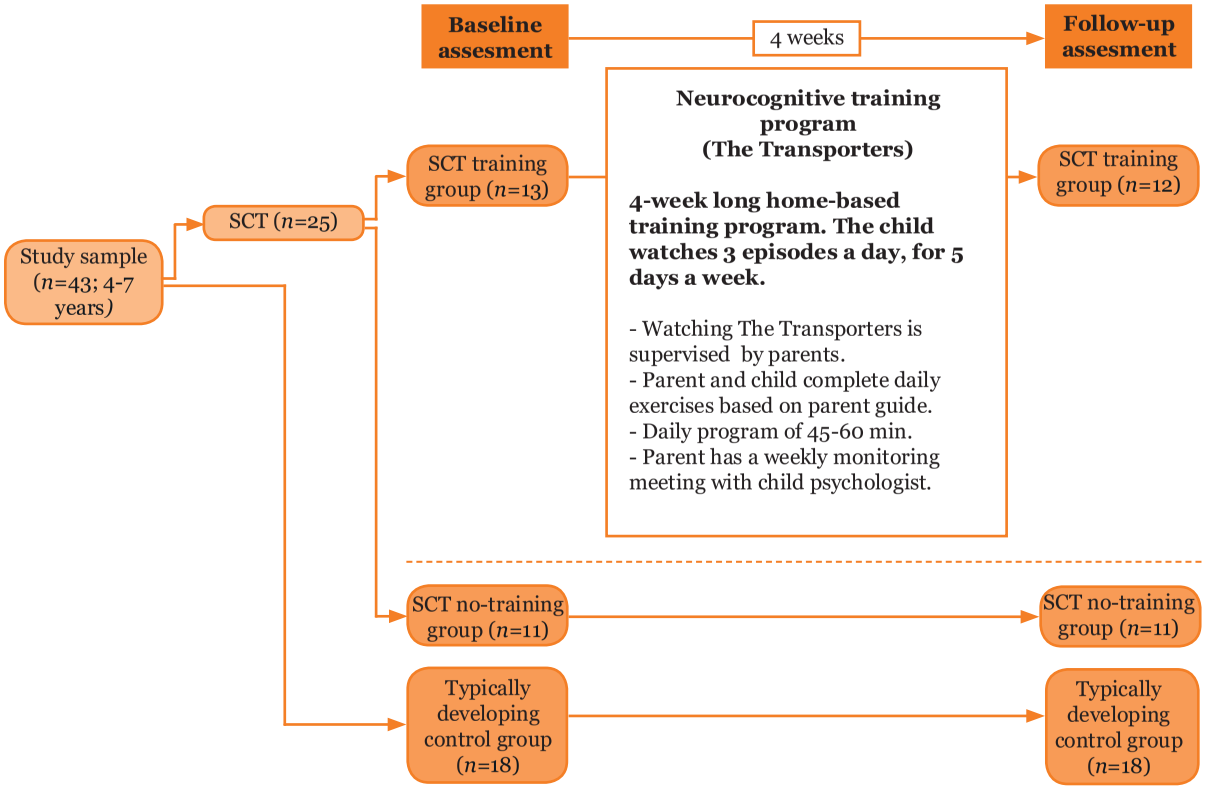


Figure 1. Flow diagram of the study design with the SCT training group, the SCT no-training group and a typically developing control group.
Note: SCT = Sex Chromosome Trisomie.

Emotion training program: The Transporters

The Transporters is a narrated and animated DVD series and was originally developed to teach young children between the age of 3 and 8 about emotions, their causes and consequences, and their corresponding facial expressions (Changing Media Development, www.thetransporters.com; (Baron-Cohen, Golan, Chapman, & Granader, 2007). The series consists of fifteen 5-min episodes, portraying key emotions including basic emotions and nine more complex emotions: happy, sad, angry, afraid, disgusted, surprised, excited, tired, unfriendly, kind, sorry, proud, jealous, joking and ashamed. The narrated stories are built around eight characteristics who are vehicles (e.g. trams, cars, railway) with real-life faces of actors showing the emotions. The emotion is presented in the context of the series plot, in which the emotions are labeled, facial expressions are highlighted, and the context of the emotional experience is provided within social interactions between the toy vehicles. The assumption behind The Transporters is that through repetitive watching of entertaining episodes children might enhance facial emotion recognition and understanding skills (see for an extensive description of The Transporters: (Golan, Ashwin, Granader, McClintock, Day, Leggett, & Baron-Cohen, 2010). The Transporters has been proven to be successful in improving emotion understanding abilities in young children with ASD (Golan et al., 2010; Young & Posselt, 2012), although mixed results were found with respect to efficacy of The Transporters in young children with ASD with a lower range of cognitive ability (Williams, Gray, & Tonge, 2012; Gev, Rosenan, & Golan, 2017). See Figure 2 for screenshots of the first episode.

Figure 2. Screenshot of an episode from The Transporters training program: character William showing the emotion 'happy'.

Children in the SCT training group watched the Dutch version of The Transporters in their home setting (De Ambelt, The Netherlands; resources.autismcentreofexcellence.org); they watched three episodes a day supervised by the parent, for 5 days a week and four weeks long (see Figure 1). The episodes were repeated in the same order every single week, in order to achieve repetitive watching of the episodes. Parents were provided with a detailed manual that consists of operating instructions, and a daily diary with a general introduction to the separate episodes, and exercises and questions to discuss with their child after watching the episodes. These exercises and questions were aimed to broaden the child's understanding of the emotional concepts as presented in the episodes, and to facilitate consolidation of learned skills. Examples of exercises and questions are: 'Who is kind to you when you are in a bad mood? What does this person do? How does that make you feel? What do you do when you see that your mother/father/brother/sister/friend is sad or worried?' During the training period, the parent had a weekly call with the researcher to discuss and find solutions for any practical bottlenecks.

Instruments

Background measures: Global level of cognitive and language development. At baseline, global level of cognitive and language development was assessed in all children. Four subtest of the WPPSI were used to estimate global level of intelligence (Block Design, Matrix Reasoning, Vocabulary, and Similarities; Wechsler, 2002). Total IQ estimates were calculated based on this short form version of the WPPSI-III (Hurks, Hendriksen, Dek, & Kooij, 2016). The Peabody Picture and Vocabulary Test (PPVT; Dunn, & Dunn, 1997) was used to measure receptive language level. To measure expressive language skills, the Clinical Evaluation of Language Fundamentals - Preschool, 2nd edition (CELF-Preschool; Wiig, Secord, & Semel, 2004) was administrated.

Facial emotion recognition. The Affect Recognition subtest of the NEPSY-II neuropsychological test battery (Korkman, Kirk, & Kemp, 2007) was used to assess children's ability to discriminate among common facial emotions from photographs of children, and was administrated at baseline and follow-up. The task has been normed with typically developing children aged 3-16 years old. During the task, participants are required to match faces of different children who show the same

emotional expressions (happy, sad, angry, disgust, fear and neutral). The participant indicates if two expressions are the same or different, determines which two faces have similar expressions, or identifies two children with expressions that match a third child's face. The total raw score range is between 1 and 35, with higher scores reflecting a better ability to recognize facial expressions.

Theory of Mind. The ToM subtest of the NEPSY-II neuropsychological test battery (Korkman, Kirk, & Kemp, 2007) was used to assess children's understanding of mental states and other people's perspectives at baseline and post-training. The ToM subtest consists of two different subtasks: verbal tasks and contextual tasks. In the verbal tasks, the questions are based on verbal scenarios with (6 items) or without (11 items) support of pictures. They measure the understanding of (false) beliefs, intentions, other's thoughts, ideas and comprehension of figurative language. Two items aim to measure the child's verbal and gestural imitation abilities, as imitation abilities are thought to be a basic ability for ToM skills. The child is asked to answer the tasks verbally, with the exception of an imitation question where the child is asked to imitate gestures or words. In all of the items the child can answer very briefly; one word is often sufficient for a correct answer, and in two of the questions it is also possible to answer by pointing. The contextual tasks of the ToM subtest aim to measure the child's ability to relate affects to a broader social context. In these items the child is shown drawings with children in social contexts. In each drawing there is a target girl whose face is not shown. The child is asked to select one of four photographs of the same girl's face with different emotions selecting the emotion of the girl in the drawing. The child can answer by pointing. The total score range is between 1 and 28 (sum score of the 15 verbal tasks and 6 contextual tasks), with higher scores reflecting better ToM skills.

Social Orienting. Eye gaze fixations towards key sources of social information (eyes, faces) were measured during a Social Orienting Paradigm (see for a detailed description of the paradigm: Bouw, Swaab, Tartaglia, & Van Rijn, 2021), at both baseline and follow-up. The 30 s. during video showed a social plot, in which social cues are reciprocally exchanged between a child and an adult. To prevent interference with language abilities, language used in the clip was not the same as the language of the participants (i.e. Italian vs. Dutch). In a group of non-clinical young children aged 3-7 years, this eyetracking paradigm was found to be related to real-life social

behaviors, and independent of age, IQ, or gender (Van Rijn, Urbanus, & Swaab, 2019). See Figure 3 for a screenshot of the video clip.

Figure 3. Screenshot of the video clip in the Social Orienting Paradigm.

Eyetracking equipment and procedures

Gaze data within specific areas of interest (AOIs) was collected using the Tobii X2-60 eyetracker (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 computer with eyetracker was placed on a table adapted to the height of the seat, and the child was seated in a car seat at 65 cm viewing distance. A 5-point calibration procedure was used, with successful calibration defined as a maximum calibration error of 1 degree for individual calibration points (i.e. < 1 cm at a distance of 65 cm from the eyetracker). After the calibration procedure, the child was instructed to watch the movie clips and pictures on the computer. The paradigm started with an attention grabber (e.g. a moving picture of an animal, shown on a black background and accompanied by a sound) to direct the attention of the child to the screen.

Gaze data was processed using Tobii Studio (version 3.2.1), using the Tobii Identification by Velocity Threshold (I-VT) fixation filter. A fixation was registered if the velocity threshold for an eye movement exceeded 30°/s, and therefore controls for validity of the raw eyetracking data making sure only valid data were used (Olsen, 2012). The 'Dynamic AOI' tool was used to draw AOIs, drawn with a one centimeter margin, to ensure that the AOIs were sufficiently large outside the defining contours to reliably capture the gaze fixation (Hessels, Kemner, van der Boomen, Hooge, 2016). Dynamic AOIs were grouped into the following categories: eyes, faces and the

whole screen. In order to evaluate the amount of nonvalid eye tracking data, the total visit duration toward the whole screen was calculated, divided by the duration of the clip, multiplied by 100, reflecting the percentage of valid data collected during each of the eye tracking tests. Proportions fixation duration were calculated by taking the total fixation duration within the AOI, divided by the total visit duration toward the whole screen of the individual child, multiplied by 100, reflecting the percentage of time children were attending to an AOI.

Feasibility of the emotion training program

Social validity parents. The Social Validity Questionnaire (SVQ; Rollins, 2013) was filled in by the primary caregiver of the children in the SCT training group at follow-up, and assessed the parent's perception of: (a) how easy the training was to incorporate into daily life; (b) how easy the training was to learn; and (c) whether the training was effective for the child and family. The SVQ consist of 15 items, and were rated on a 5 point Likert scale ranging from 1 'Totally disagree' to 5 'Totally agree'. Examples of items are: 'This training was easy to incorporate into my family life', 'This training was not complicated to learn' and 'I noticed meaningful increases in my child's social interaction with the people in his environment.'

Perception of children about the training. Children in the SCT training group reported how much they liked the training episodes in a daily diary. Their perception about the training was rated on a Visual Analogue Scale (VAS) at a daily basis on an interval from 0-100 (see Figure 4). According to Shields, Palermo, Powers, Grewe, & Smith (2003), only responses that were properly marked on the VAS lines (the mark must be a single vertical line that is no more than 1 mm away from the VAS line) and responses that were marked along the entire length of the VAS line (as opposed to using just the end-points and/or the middle of the VAS line) were used in the analysis. A total score was computed for each child by taking the sum of the registrations divided by the number of registrations (with a maximum of 20 registrations). Furthermore, how much children liked the training was calculated for every single week.

Figure 4. Visual Analogue Scale used during the training program.

Study procedures

Assessments ate baseline and follow-up took place in a quiet room at the university or at home. Administration of the WPPSI, PPVT, CELF and NEPSY was performed on a table by trained child psychologists. The eyetracking procedure took place after the neurocognitive tests administration. The laptop with the eyetracker was placed in a small tent to standardize the testing environment, and to control for light conditions. The child was seated in a car seat in front of the eyetracker. The examiner was seated beside the child (directing Tobii Studio with a remote keyboard), and started the calibration procedure. Parents were allowed to stay in the room (out of sight) and were asked not to communicate with their child during the procedure. The Social Validity Questionnaire was filled in by the primary caregiver of the child.

Ethical approval and informed consent

This study was approved by the Ethical Committee of Leiden University Medical Center, The Netherlands. Signed informed consent was obtained from the parents of all participating children, according to the declaration of Helsinki.

Data analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25. Baseline difference between study groups on background measures (global cognitive and language level) were analyzed with ANOVAs. In order to analyze the training effects, Repeated Measures MANOVAs were used with Time (baseline, follow-up) as within variable and Group (SCT training, SCT no-training, typically developing) as between variable. The interaction effect (Time x Group) was used to evaluate overall training effects. Post-hoc paired sample t-tests were carried out to analyze change in social cognitive abilities within the three study groups (SCT training, SCT no-training, typically developing). Repeated Measures MANCOVAs

were carried out to test training effects, while covarying for cognitive and language abilities. Change in reported perception of children about the training was analyzed with a RM ANOVA. Level of significance was set at $p < .05$, two-tailed. Effect sizes were calculated with Cohen's *d* or partial η^2 when applicable.

Results

Background measures

Mean scores on cognitive background measures at baseline (global cognitive level, receptive and expressive verbal ability) are presented in Table 2. The three study groups do not differ on expressive verbal ability ($F(2,39) = 2.35$, $p = .108$). However, the groups significantly differ in global cognitive functioning ($F(2,39) = 7.64$, $p = .002$), indicating lower functioning in the SCT groups (training and no-training), compared to the TD group. Both SCT groups perform similar on global cognitive functioning. Also, a significant difference on receptive verbal ability is found ($F(2,39) = 5.39$, $p = .009$), indicating lower ability in the SCT no-training group, compared to the TD group. No difference between the SCT training group and the no-training group was found for receptive verbal ability. Because of these differences between the SCT groups and the TD group, global cognitive functioning and receptive verbal ability are added as covariates in the analyses.

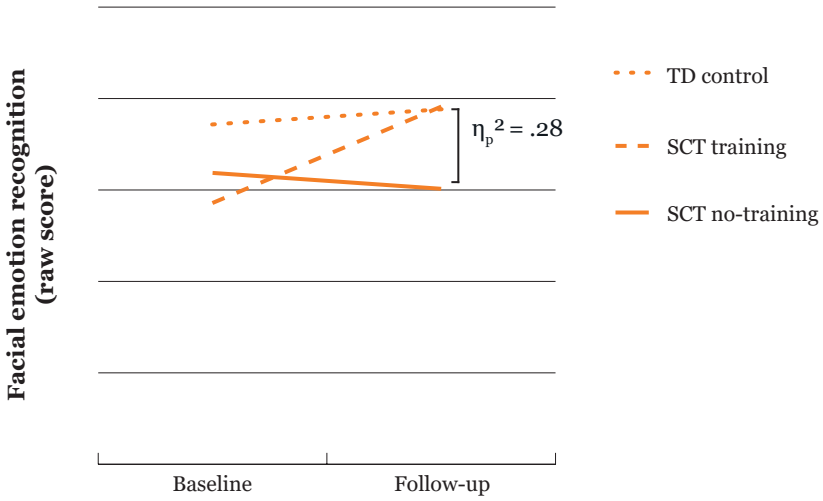
Table 2. Means (SD's) and group differences on cognitive and language functioning.

	SCT training	**SCT no-training**	**TD control**	*p*-value	Effect size (η_p^2)	Group differences
	n = 13	*n* = 11	*n* = *18*			
Global cognitive functioning standard score; WPPSI-III	92.77 (10.68)	91.09 (14.98)	107.78 (13.20)	.002	.28	A, B < C
Receptive verbal ability standard score; PPVT-III	103.00 (11.10)	97.82 (12.33)	111.39 (10.55)	.009	.22	B < C
Expressive verbal ability scaled score; CELF	9.31 (2.66)	8.36 (2.87)	10.33 (1.85)	.108	.22	

Note: SCT = Sex Chromosome Trisomies; TD = typically developing. A = SCT training group; B = SCT no-training group; C = typical developing control group.

Training effect: Facial emotion recognition

First, to evaluate the overall effect of the training a RM MANOVA is conducted with Time (baseline, follow-up) as within variable and Group (SCT training, SCT no training, typically developing) as between variable. The analysis yield a significant interaction effect for Time x Group (F (2, 39) = 7.50, p = .002, η_p^2 = .28), with a large effect size. This significant interaction effect on emotion recognition skills remains, even when global intelligence and receptive language skills are added as covariates (F (2, 37) = 6.65, p = .003, η_p^2 = .26). Next, post-hoc paired sample t-tests are used to analyze the effect of Time within the three study groups. In the SCT training group, a significant change in emotion recognition abilities is found (t (12) = -3.72, p = .003). In the SCT no-training group, no significant change in emotion recognition is found (t (10) = 0.88, p = .401), neither in the typically developing group (t (17) = -0.88, p = .393). These findings indicate a significant change in emotion recognition abilities in the SCT training group, that is not present in the SCT no-training group or typically developing control group. After completing the training, the SCT training group (M = 19.54, SD = 4.72) scores comparable to their typically developing peers (M = 19.39, SD = 4.98; t (29) = 0.84, p = .933). In terms of standard deviations, children in the SCT training group score 1.11 SD higher as compared to their average baseline score. See Figure 5 for an illustration of the interaction effect on facial emotion recognition.



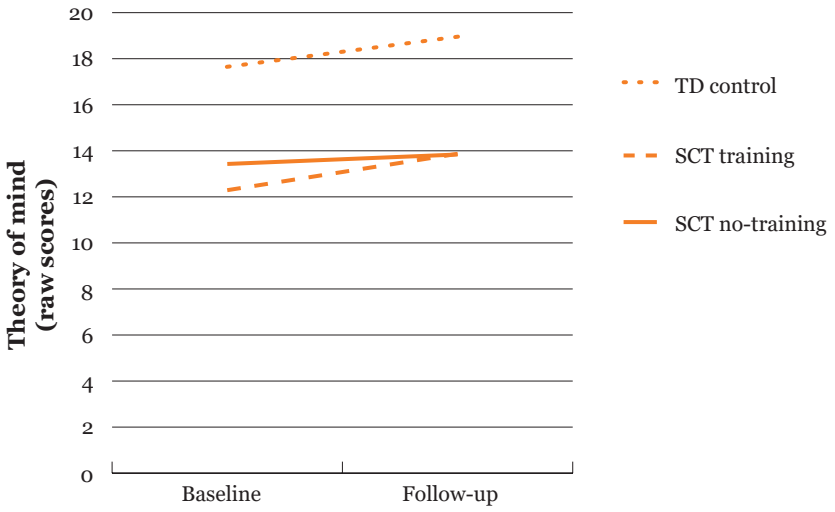
Emotion recognition	*M (SD)* baseline	*M (SD)* follow-up	Δ-value	*p*-value	Cohen's *d*
SCT training	14.31 (5.17)	19.54 (4.72)	5.23	.003	1.06
SCT no-training	15.91 (6.72)	15.09 (7.13)	-0.82	.401	0.12
TD control	18.61 (4.89)	19.39 (4.98)	0.78	.393	0.16

Figure 5. Training effect on facial emotion recognition: mean scores before (Baseline) and after completing the training (Follow-up).

Note: SCT = Sex Chromosome Trisomies; TD = typically developing.

Training effect: Theory of Mind

First, to evaluate the overall training effect on Theory of Mind, a RM MANOVA is conducted. No significant interaction effect is found for Time x Group (*F* (2, 39) = .31, *p* = .738) indicating no training effect on Theory of Mind. These findings do not change when global intelligence and receptive language skills are added as covariates (*F* (2, 37) = 0.73, *p* = .488). See Figure 6.



Theory of Mind	*M (SD)* baseline	*M (SD)* follow-up	Δ-value	*p*-value	Cohen's *d*
SCT training	12.31 (5.38)	13.85 (6.40)	1.54	.114	0.26
SCT no-training	13.45 (6.46)	13.82 (5.76)	0.37	.826	0.06
TD control	17.67 (4.24)	18.94 (5.01)	1.27	.097	0.27

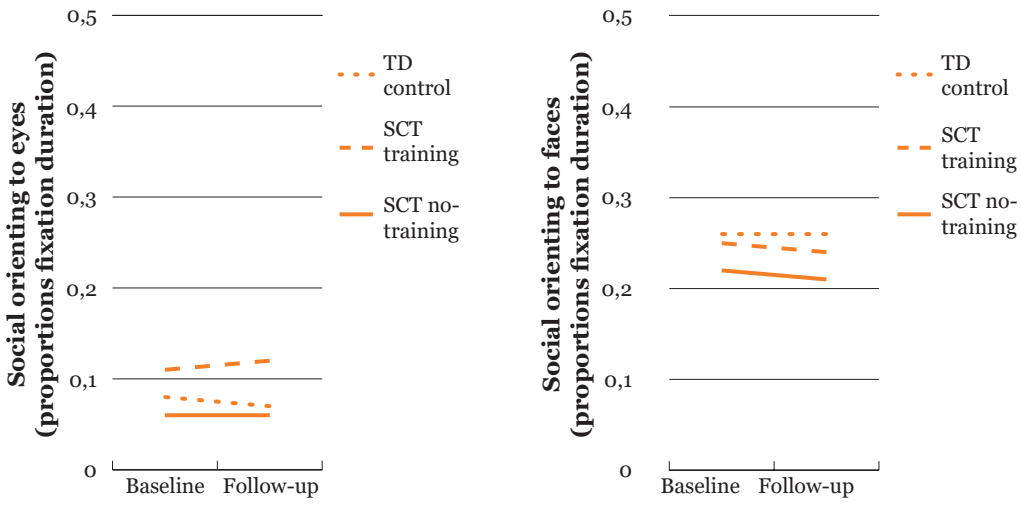
Figure 6. Theory of Mind (total scores) before (Baseline) and after completing the training (Follow-up).
Note: SCT = Sex Chromosome Trisomie; TD = typically developing.

Training effect: Social Orienting to eyes and faces (eyetracking paradigm)

Attention to the screen. The Social Orienting Paradigm was successfully completed by 42 children at baseline, and 41 children at follow-up (one boy with 47,XXY in the SCT no-training group was not able to complete the task at follow-up). At baseline, the total proportion valid on-screen fixation duration is 95.5%, indicating sufficiently high attention to the screen. Attention to the screen do not significantly differ between the three study groups, F (2, 39) = 1.76, p = .186). Similar at follow-up, the total proportion valid on-screen fixation duration is 94.3%, and do not significantly differ between the three study groups, F (2, 38) = 1.76, p = .186).

Training effect. A RM ANOVA is conducted to analyze overall training effect, revealing no significant effect of Time x Group for social orientation to eyes (*F* (2,

38) = 0.22, p = .803) neither to faces (F (2, 38) = 0.05, p = .948). These findings do not change when global intelligence and receptive language skills are added as covariates (eyes: F (2, 36) = 2.15, p = .131; faces: F (2, 36) = 0.29, p = .741). See Figure 7.



A. Social orienting to eyes % fixation duration	***M (SD)* baseline**	***M (SD)* follow-up**	**Δ-value**	***p*-value**	**Cohen's *d***
SCT training	.11 (.23)	.12 (.26)	.01	.468	0.04
SCT no-training	.06 (.05)	.06 (.06)	.00	.758	0.00
TD control	.08 (.08)	.07 (.04)	-.01	.832	0.16

B. Social orienting to eyes % fixation duration	***M (SD)* baseline**	***M (SD)* follow-up**	**Δ-value**	***p*-value**	**Cohen's *d***
SCT training	25 (.10)	.24 (.09)	-.01	.773	0.11
SCT no-training	.22 (.12)	.21 (.10)	-.01	.448	0.09
TD control	.26 (.09)	.26 (.11)	-.00	.931	0.00

Figure 7. Social orienting to eyes (A) and faces (B) before (Baseline) and after completing the training (Follow-up).
Note: SCT = Sex Chromosome Trisomy; TD = typically developing.

Feasibility of the emotion training program

Social validity parents. Response on the 5-point Likert scale of the 15 items of the Social Validity Questionnaire are recoded into three categories: negative opinion (value 1 and 2), a neutral opinion (value 3), and a positive opinion (value 4 and 5). Table 3 presents parents' responses on the three subscales of the SVQ. In sum, parents report positive experiences after the training period. To illustrate, 91.7% of the parents report that the training is easy to implement in daily life, 66.7% reported that the training is easy to learn and to use and valuable for their child. Although 83.3% of the parents recognize positive changes in their child after intervention (Q11: This intervention provided a significant positive change for my child), a vast majority of the parents are neutral about the generalization of the intervention to other contextual situations (Q12: I noticed meaningful increases in my child's interaction with the people in his environment; Q15: Other people noticed a significant positive change in my child). Only 8.3% of the parent report positive increases in child's eye contact (Q14: I noticed meaningful increases in my child's eye contact with the people in his/her environment). Parents do report that they would recommend the intervention to other parents and that they are willing to continue using the intervention model in the future. See the supplementary material (Table 1) for parents' response on all items of the SVQ.

Table 3. Social validity of parents in the SCT training group on subscales of the Social Validity Questionnaire.

	Negative opinion	Neutral opinion	Positive opinion
	strongly disagree/disagree	neutral	agree / strongly agree
Incorporation in daily life	8.3%	0%	91.7%
Easiness to learn the training	0%	33.3%	66.7%
Effectiveness of the training	16.7%	58.3%	25.0%

Note: N=13

Perception of children about the training. Children were asked daily how much they liked the training, on a Visual Analogue Scale. The mean VAS-score of children during watching the training episodes for the SCT training group is 65.2 on a scale of 0-100. There is no significant difference in VAS-scores of children reported between the first training week (M= 73.40, SD= 26.62), the second week (M= 64.76, SD=

30.31), the third week (M= 63.67, SD= 29.17), and the fourth week (M= 59.43, SD= 30.07), F (3,24) = 1.70, p = .194 (see Figure 8).

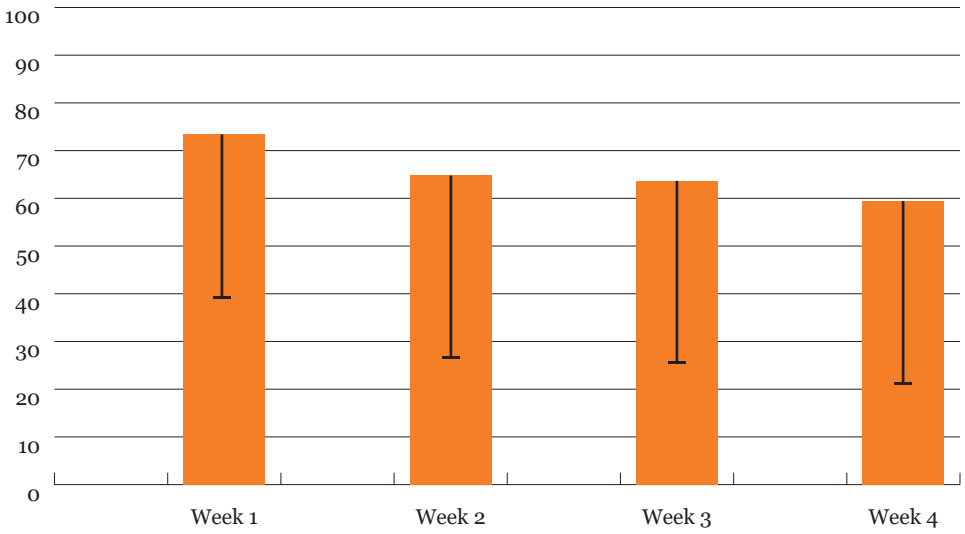


Figure 8. Perception about the training during the training program, reported by children on a Visual Analogue Scale (VAS).
Note: ⊣ = SD (only lower bar depicted)

Discussion

There is a great need for evidence-based interventions that support early development of young children with Sex Chromosome Trisomies (SCT). The current study aims to evaluate the effectiveness of a neurocognitive training in young children with SCT, aged 4-8 years. As social cognitive and behavioral vulnerabilities have been identified as among the key areas of vulnerability in SCT (Urbanus, Swaab, Tartaglia, Cordeiro, & Van Rijn, 2020; Van Rijn, 2019), this neurocognitive training was targeted at improving the understanding of social cues from facial expressions.

Efficacy of the training was examined on key aspects of early social cognition that have proven to be vulnerable in young children with SCT: facial emotion recognition, Theory of Mind and social orienting (Bouw, Swaab, Tartaglia, & Van Rijn, 2021). Three study groups were included in the study: 4-8 year old children with SCT, and two age- and gender-matched control groups that did not complete the training: one

waiting list group with SCT and one typically developing group. Promising results regarding the effectiveness of the training were found, revealing that attending the 4-week home based neurocognitive training was effective in significantly improving the ability to identify and match basic and complex facial expressions in children with SCT, with a large effect size. These findings were irrespective of level of global cognitive functioning and expressive and receptive language abilities. After completing the training program, children with SCT show emotion recognition abilities to a level that could not be distinguished from the typically developing group at follow-up.

These findings illustrate that there are opportunities for positively supporting the development of emotion understanding in children with SCT, already at a young age. Given the evidence that in SCT early social cognitive vulnerabilities may emerge and present more profoundly with age (Bouw, Swaab, Tartaglia, & Van Rijn, 2021), early support of early social cognitive development may alter adverse developmental trajectories of young children with SCT, reduce the negative long-term impact of SCT on social adaptive functioning (Mundy & Neal, 2001; Dawson, 2008).

Improvements in facial emotion recognition were measured with a standardized task which required understanding of facial emotion of real human faces, different from the learned emotions attached on animated vehicles (Korkman, Kirk, & Kemp, 2007). Also, this standardized task gave no information of the emotion in terms of its context, supporting the notion that children with SCT were able to generalize their acquired knowledge during the training program on a distant generalization task. This is especially interesting, as other neurocognitive training programs aimed to enhance emotion recognition in other populations, often show limits in the generalization that were possible to achieve (see for example in ASD populations: (Golan & Baron-Cohen, 2006; Bölte, Feineis-Matthews, Leber, Dierks, Hubl, & Poustka, 2002). The found training effects in the present study were independent of children's global cognitive functioning and their expressive or receptive language skills which are proven to be lower in young children with SCT (Urbanus, Swaab, Tartaglia, Boada, & Van Rijn, 2021), suggesting that neurocognitive training programs may be suitable and effective for a broad range of young children with SCT.

There were also areas of social cognitive functioning that did not change following the neurocognitive training program, as the study findings indicate that increased

emotion recognition abilities after the training did not generalize to direct improvements in social orientation or Theory of Mind. These findings indicate a specific effect of the training on emotion recognition abilities which was the target ability to be trained in the program (Golan et al., 2010). However, the findings of the current study suggest that The Transporters training program is effective in training understanding of facial emotion in young children with SCT, rather than being effective in enhancing broad early social cognitive development.

Lastly, this study found positive parent and children's perceptions on the feasibility of the training program. The Transporters is an intensive program, expecting the child and parent to invest 45 to 60 minutes (i.e. 3 videos accompanied with the exercises from the parent guide) 5 days a week, for a total duration of 4 weeks. Nonetheless, parents were positive about the ability to incorporate the training program in daily life and report that The Transporters was easy to learn and easy accessible. These findings indicate that intensive involvement and guidance of parents during the training program is feasible, which has proven to be effective in generalization and maintenance of learned emotion recognition skills (Gev, Rosenan, & Golan, 2017). In addition, our findings indicate an intrinsic motivation of children in the SCT training group to watch the animated series, as they reveal that children on average liked the training program from the beginning until the end. These results support the assumption that The Transporters training use intrinsically motivating animated media in a way that children like watching the episodes while learning about emotions in their context (Golan et al., 2010).

The current study was the first one, to our knowledge, to explore the effectiveness of a neurocognitive training in children with SCT. The inclusion of a training group and two control groups (a SCT waiting list group and a typically developing group), ensured the possibility to check for natural increases or learning effects in social cognitive functioning. Although further research is needed, the current results may contribute to improving clinical care in order to prevent negative long-term impact of SCT on social (cognitive) development. As neurocognitive training programs are easy accessible, cost-effective, and can be used without a clinical indication, support of early neurocognitive development can be preventively executed in home-based and school-based settings. Neurocognitive training programs can also be used as part of an integrative intervention program for young children with SCT at risk

of specific social cognitive vulnerabilities, which have become visible based on individual neurocognitive assessment.

While the results of the present study are promising, future research is needed to address its limitations. First, the small sample size of this study especially when it comes to boys with 47,XYY limits the generalizability of the findings. Because of these small samples, we were not able to assess the specific contribution of karyotype (XXX, XXY, XYY) on the efficacy of the training program. Second, the present study only assessed post-training outcome, and did not have a follow-up period to investigate maintenance of the improved abilities and possible longer-term generalization effects. It remains for future studies to evaluate how support of early social cognition is related to functional outcomes, in order to prevent the detrimental impact of the presence of SCT. Replication is therefore necessary in future research, preferable in Randomized Control Trials studies with larger samples sizes and follow-up maintenance assessments, in order to investigate specific effects of neurocognitive training programs within the different karyotypes and longer term training effects. Another promising approach that could be used as a research method complementary to RCTs is the Single Case Experimental Design (SCED; Maric, de Haan, Hogendoorn, Wolters, & Huizenga, 2015), the appeal of case-based time-series studies, with multiple assessments both before and after intervention. Benefits of SCEDs include the possibility to investigate the efficacy of early intervention in heterogeneous populations (e.g. populations with highly variable phenotypes such as SCT), and being able to test the effectiveness of treatment methods in the complex but real world practice of clinical work.

Taken together, the current study on the efficacy of a neurocognitive training in young children with SCT, an animated facial emotion training program, showed a significant improvement in facial emotion recognition abilities, with a large effect size. Moreover, encouraging results were found with respect to parents' and children's perception on the feasibility of the training program. These findings indicate that it is possible to (preventively) support the development of social cognition in children with an extra X- or Y-chromosome, which may reduce the negative long-term impact of SCT on social adaptive functioning. Additional research is warranted using a larger sample and follow-up maintenance assessments in order to further evaluate the effectiveness of the training for specific subtypes of SCT. Evidence based support

of young children with SCT is of great importance given the need for preventive and early training programs, aimed to minimize neurodevelopmental impact.

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CHAPTER 8

General discussion

The aim of the present thesis was to investigate the impact of SCT (XXX, XXY, XYY) on early social adaptive functioning and underlying social cognitive mechanisms, and to study the effectiveness of early preventive support, targeting social cognitive functioning. Specific effects of the extra X and Y chromosome on neurobehavioral development have been found in domains of social adaptation, self-regulation and language (Van Rijn, 2019; Urbanus, Van Rijn, & Swaab, 2020; Tartaglia et al., 2020). SCT is consequently associated with increased risk for symptoms of Attention Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorders (ASD; van Rijn, 2019). Due to advances in noninvasive prenatal testing technology, the number of prenatal diagnoses of SCT is rapidly increasing (Tartaglia et al., 2020). Given this, there is the opportunity to prospectively investigate early developmental pathways towards specific vulnerabilities in children, adolescents and adults with SCT, before any adversities in daily functioning of the genetic condition occurs. In that way, genetic conditions such as SCT serve as naturalistic 'at risk' models of neurodevelopment, because they are associated with increased risk for neurobehavioral difficulties and psychopathology (Reiss et al., 2000). Identifying vulnerabilities during early social development in genetic conditions such as SCT, may thereby give insights in etiological pathways leading to complex behavioral phenotypes.

Because of the rise in prenatal diagnoses of SCT, there is not only the opportunity to prospectively investigate early development, but also the great need to gather knowledge on the early development of children with SCT. Insights in early development will help to identify targets for early monitoring and early intervention, to develop and inform evidence-based care in order to improve health and neurodevelopmental outcome for this growing population of early identified children with SCT. We therefore aimed to study early social adaptive functioning and underlying social cognitive mechanisms in a relatively large and international group of children with SCT, aged 1-8 years old.

Research questions and design

Previous studies on the impact of SCT on social adaptive functioning have focused on populations with broad age-ranges, including participants from middle childhood to adulthood. In these studies, it was found that social adaptive functioning is among the key domains of vulnerability in SCT (Van Rijn, 2019; Urbanus, Van Rijn, &

Swaab, 2020; Tartaglia et al., 2020). Social adaptive development is characterized by the acquisition of social abilities, referring to a spectrum of functions that allow an individua to interact and communicate with others, and that are crucial for reciprocal and lasting relationships and for participating in society (Beauchamp & Anderson, 2010). In typical development, these social abilities develop gradually in a temporal sequence of social milestones that may be needed to shape appropriate and adaptive social functioning (Soto-Icaza, Aboitiz & Billeke, 2015). In the present thesis, we firstly focused on the possible impact of SCT on early social behavioral abilities (e.g. early social communicative abilities such as making eye contact and the initiating of responsive interactions), and on how SCT possibly impacts the way children shape social interactive behavior under varying levels of social load during social interactions.

Second, we addressed the question how SCTs impact social cognitive mechanisms underlying social behavior. Social cognition is defined as the ability to perceive, and understand social signals, and to adequately react in social interactions (Crick & Dodge, 1994). In the first years of life, social cognitive development is characterized by the interactive emergence of several essential elements, such as eye gaze to faces and emotion recognition. In the present thesis, we studied the impact of SCT on social attentional mechanisms (i.e. eye gaze to faces and social orienting), shared social attention (i.e. joint attention), the understanding of emotions from facial expressions and Theory of Mind.

Third, we investigated the severity of social vulnerabilities in young children with SCT, by studying whether SCT is associated with the appearance of symptoms that are typical in Autism Spectrum Disorder (ASD). We also questioned how an early social cognitive marker (i.e. joint attention) in young children with SCT may be related to social impairment later in life, as expressed in symptoms of ASD.

Given the rise in prenatal diagnoses of SCT, there is the urgent need for knowledge about the question whether early preventive interventions may possibly reduce risk for difficulties in adaptive functioning and psychopathology later in life (Herlihy & McLachlan, 2015). Therefore, we lastly focused on the possible effects of a neurocognitive training program to improve a core aspect of social cognition, namely the understanding of social cues from facial expressions. We studied the efficacy of

this program in 4-8 year old children with SCT.

The findings described in the present thesis are part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study – Leiden, The Netherlands), which includes children with SCT and nonclinical controls aged 1-8 years. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. We studied more than 100 children with SCT aged 1-8 years and an age-matched typical developing group of 100 children. By identifying the effect of specific karyotype-subtype and possible recruitment bias, we were able to identify phenotypic differences within the SCT group.

The design of the studies presented in the current thesis allows us to investigate early and prospective social development in SCT, and will help to identify early targets for monitoring and early intervention, leading to improved clinical care and probably to better developmental outcomes for young children with SCT. Recruitment and assessment took place at two sites: the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, The Netherlands, and the eXtraordinary Kids Clinic at Children's Hospital Colorado in Denver, USA. The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping.

Summary of main results

The results in this thesis reveal an impact of SCT on social adaptive and communicative behavior at a very early age, expressed in difficulties with responding and initiating early social communication and in daily life social emotional development (Chapter 2). We also found more social withdrawal during observed social interactions in a structured play situation in children with SCT, aged 1-8 years. Interestingly, we found that social withdrawal is more pronounced when social load in the interaction is high, meaning that social input and demands from the environment are conditional for the formation of social behavior in interaction with the social environment (Chapter 3). To explore the extent to which early social vulnerabilities are associated with symptoms that are typical in ASD, we examined the possible impact of SCT on the early appearance of ASD symptoms. The results demonstrate that ASD symptoms are substantially higher in children with SCT compared to the general population. A

significant finding includes that an impact of SCT was especially found on domains of social interaction and communication. In the sample of the present thesis, 22% of the children with SCT were at clinical risk for a clinical diagnoses of ASD, including restricted interests and repetitive behaviors (Chapter 3 / Chapter 6). Joint attention, a pivotal dimension of infant social cognition that serves as an important milestone in typical social development, showed to be predictive of severe social impairments reflected in ASD symptoms in children with SCT at one year follow-up (Chapter 6).

Chapter 4 and 5 provide evidence for an impact of SCT on early social cognitive mechanisms underlying social adaptive behavior. By using eyetracking, we found an impact of SCT on basic social cognitive mechanisms of social attention to faces and eyes, social orienting and joint attention, indicating that children with SCT are less inclined to visually orient towards social information and have difficulties with following gaze and point gestures of a social partner. Also, an impact of SCT on more complex and specialized social cognitive abilities was found: young children with SCT showed vulnerabilities in the ability to understand emotions from facial expressions. Similar, substantial difficulties with understanding mental states of others (i.e. Theory of Mind) were found in young children with SCT. These findings suggest that social behavioral difficulties may be anchored in altered perceiving and processing of social information already early in neurodevelopment.

Interestingly, a 'growing into deficit' phenomenon (e.g. Rourke et al., 2007; Sprong, 2008) was found for early social cognitive mechanisms: the phenomenon of an increasing deviation of abilities as compared to typical developing peers. And finally, a significant effect of a preventive neurocognitive training on emotion recognition abilities was found in 4-8 year old children with SCT, suggesting that there are opportunities for positively supporting the development of social cognition in children with an extra X or Y chromosome (Chapter 7). For the key findings of the present thesis, see Box 1.

In this chapter, the main results of the six studies are discussed in more detail, followed by directions for future research and implications for clinical practice, concluded by a summary of the main findings.

Box 1. Key findings of the present thesis.

Key points

* SCT impacts social behavioral development from the first years of life, reflected in vulnerabilities in early social communication and social interaction. Social interaction vulnerabilities include lower frequencies of eye contact, more difficulties with non-verbal expressions of desires/beliefs, responding during social interactions and more social withdrawal, especially when demands from the environment were high.
* Early social cognitive dysfunctions that may underlie challenged social development in SCT include visual orientation to social cues, joint attention, facial emotion understanding and Theory of Mind.
* Early social cognitive development in SCT is characterized by a 'growing into deficit' phenomenon.
* Children with SCT are at increased risk for symptomatology associated with Autism Spectrum Disorder, with 22% of the children reaching clinical thresholds on both the domains of social interaction and communication and restricted interests and repetitive behavior.
* Joint attention is predictively related to symptoms of ASD at one year follow-up, therefore serving as an early marker for children with SCT at risk for severe social impairments.
* Neurocognitive training of emotion recognition abilities has the potential to mitigate impact of the extra X or Y chromosome on early social cognition.

Discussion of main results

Early social and communicative behavior in SCT

Based on our findings we can conclude that, already very early in development, i.e. at the age of 1 year, children with SCT have vulnerabilities in the ability to socially communicate with others and that they often suffer from daily live social emotional dysfunction (Chapter 2). We used systematic behavior observations to explore social communication behaviors in 12-24 months old children with SCT when they are

actually exposed to social interactions in a structured play situation. These structured play observations showed that, on average, 12-24 months old children with SCT display lower frequency of eye contact in responding to social communication, as compared to their peers. Furthermore, the results indicate that 12-24 months old children with SCT have difficulties with early social communication, like joint attention, non-verbal expressions of desires and/or beliefs, and reciprocal social interactions. The results also showed that social communication deficits in young children with SCT extend to social emotional dysfunction in daily life settings as reported by parents. It was found that, on average and compared to age-matched peers, children with SCT have more difficulties showing some typical socio-emotionally behaviors, such as showing imitations of familiar make-believe play and emotions in a back and forth way.

From a developmental perspective, it is known that vulnerabilities in early social communication and emotional functioning have a high impact on further social development (Mundy & Newell, 2007). And indeed, beside the impact of SCT during the second year of life (12-24 months) as found in Chapter 2, we also found an impact of SCT on social adaptive functioning in older age groups (Chapter 3). Expanding social interaction abilities are necessary for developing the capacity to deal with more complex social information, as for example with more social information (Soto-Icaza, Aboitiz, & Billeke, 2015). We were therefore interested whether and how SCT possibly impacts the way children shape social interaction under varying levels of social load. In general, the results revealed that children with SCT, aged 1-8 years old, displayed less instances of social engagement as compared to their age matched peers, i.e. they showed less interaction behavior and more independent play. Interestingly, social interaction and withdrawn behavior differed as a function of social load: young children with SCT showed less social interactions when social load was absent, and more social withdrawal when social load was high, as compared to their peers. Social impairments were most pronounced when the level of social load was high, as children with SCT show less interactions and more severe social withdrawal (i.e. no observable or self-stimulating behavior) in the high social load condition. These findings indicate that social input and demands from the environment are conditional for the formation of social behavior in interaction with the social environment, as the results showed that children with SCT may have difficulties with actively coping with varying levels of environmental social load and

with responding to complex social information (i.e. information with a high social load).

These findings add to the existing knowledge that an impact of SCT on social development can already be found during the first years of life, and that social interactive abilities are dependent on the complexity of social information that need to be processed. Social behavioral vulnerabilities show to be independent of age, which may suggest a stable impact of SCT on social behavioral functioning during early development.

SCT and symptoms of Autism Spectrum Disorder (ASD)

The severity of social behavioral vulnerabilities in school-aged children, adolescents and adults with SCT is illustrated by an increased level of symptoms and clinical diagnoses of Autism Spectrum Disorder (see for a review: Van Rijn, 2019). Previous studies determining the impact of SCT on ASD symptomatology have focused on populations with broad age-ranges, including participants from middle childhood to adulthood. Compared to a worldwide prevalence rate of ASD of 0.6% in the general population (Elsabbagh et al., 2012) the prevalence of ASD has been shown to be seriously higher in SCT. As SCT can be identified as early as prenatally in contrast to ASD, early social developmental pathways can be studied prospectively, before any adversities in daily functioning of the genetic condition occurs.

Risk for ASD symptomatology in genetic conditions such as SCT can be measured as the possible impact of SCT on a range of social behavioral competences that are normally distributed in the general population (Constantino & Gruber, 2012). Another approach is to study whether young children with SCT meet full ASD criteria, including pathological symptoms of clinically relevant social impairments. In the present thesis, we used both approaches in order to study the possible impact of SCT on the appearance of severe social impairments typical of ASD.

First, when considering dimensional measures of ASD symptoms, we investigated the impact of SCT on a continue spectrum, namely on domains of social awareness, social cognition, social communication, social motivation and restricted interests and repetitive behaviors during the first eight years of life (Chapter 3). We found

an increased risk for significantly elevated social impairments for all the measured dimensions: social awareness, social cognition, social communication and social motivation; young children with SCT also show significantly more restricted interests and repetitive behaviors as compared to their age matched peers. Furthermore, we also found that ASD symptoms are substantially more frequent in children with SCT compared to the general population, with a percentage of 22% of the children reaching thresholds of clinically relevant ASD symptoms (Chapter 6).

Together, these results show an impact of SCT on the appearance of severe social impairments already early in life, reflected in ASD symptomatology. The results also show that SCT mainly impacts abilities in the social and communicative domain. A subgroup of young children with SCT, namely 22%, shows the full conglomerate of ASD symptoms, including pathological symptoms of restricted interests and repetitive behaviors. Considering the impact of severe social impairments on interpersonal communication and the development and maintenance of satisfying relationships with others (Rao, Beidel, & Murray, 2008), these early ASD symptoms may indicate an 'at risk' social pathway of a considerable percentage of children with SCT. It would be interesting to further study the interaction between neurocognitive strengths and weaknesses involved in the appearance of these pathological symptoms in this specific and vulnerable group of children with SCT, for example whether and how interactions between vulnerabilities in self-regulation and executive functioning are involved in the severe outcomes of this group of children on the social domain (Borsboom & Cramer, 2013).

Cognitive mechanisms of social behavior: the impact of SCT on early social cognition

To understand mechanisms underlying impairment of social adaptation it is of great relevance that the presence of an additional X or Y chromosome is known to convergently impact the maturation of brain functions and networks involved in social adaptive cognitive and behavioral development, often referred to as the 'social brain' (Hong & Reiss, 2014; Raznahan et al., 2016). Social situations are rich in providing large amounts of information that need to be processed quickly and simultaneously. These situations trigger social cognitive mechanisms in individuals to select information in order to be able to respond adequately. Social cognition

involves the cognitive information processing mechanisms that enable us to perceive, understand social information and to interact with the social environment (Beauchamp & Anderson, 2010), and are central to interpersonal communication, to the development and maintenance of satisfying relationships with others (Rao, Beidel, & Murray, 2008), and are associated with quality of life (De Vries & Geurts, 2015). In order to understand how social cognitive development underlie social vulnerabilities in SCT, we investigated the possible impact of SCT on early social cognitive functions, and age related dynamics during early development.

Eye gaze to faces and social orienting in SCT. First of all, we focused on the possible impact of SCT on basic social cognitive skills typically developed during the first years of life, i.e. eye gaze to social information with the help of eyetracking measures. We found an impact of SCT on eye gaze patterns to social relevant information such as faces and especially eyes (Chapter 5). These difficulties with attending to social information are most pronounced in children aged three years and older, and when the social load of stimuli was high (i.e. included multiple faces). These differences between children with SCT and their age-related peers with regard to eye gaze to social information, are also found in a eyetracking measures of a social plot in which social cues are reciprocally exchanged between two persons, i.e. social orienting (van Rijn, Urbanus, & Swaab, 2019). These eyetracking results show that children with SCT at the age of 3 years had reduced visual orienting towards social cues (i.e. faces and eyes) as compared to peers. No impairments in eye gaze to social important information were seen in children in SCT aged one and two years old (Chapter 4). These impairments in visual orientation to social relevant information already early in life may deprive a child of social input needed to process social information, which in turn could disrupt brain development and social cognitive development (Mundy & Neal, 2000).

Joint attention in SCT. We also found vulnerabilities in the ability to share social attention, i.e. joint attention. Joint attention is defined as the capacity to coordinate attention between interactive social partners with respect to objects or events in order to share an awareness of the objects or events (Nation & Penny, 2008). Joint attention is associated with the early emergence of children's awareness that others have intentions, and is crucial for developing perspective-taking skills but also for other cognitive skills such as the acquisition and development of language. In young

children with SCT, we found differences in the accuracy to spontaneously follow gaze and point gestures between the children with SCT and typically developing controls, indicating difficulties with joint attention in children with SCT. These difficulties in Joint attention are irrespective of age (Chapter 4).

Facial emotion recognition and Theory of Mind in SCT. In typical development, earlier levels of social development such as social attention and joint attention develop during a temporal sequence in more complex social cognitive abilities, such as the recognition of emotions from facial expressions and Theory of Mind (Soto-Icaza, Aboitiz, & Billeke, 2015). The recognition of facial expressions gives the opportunity to detect the emotional states of others, and is therefore important during social interactions (Grossmann & Johnson, 2007). Typically from the age of three onwards the understanding of the complexity of social interactions that lead to another person's false beliefs develop (Wellman, Cross, & Watson, 2001; Devine & Hughes, 2014). This increasing comprehension of false beliefs is represented in the levels of Theory of Mind, such as the level that refers to the understanding of second-order beliefs and the recognition of influence of earlier experiences on mental states (Wellman, 2014).

In young children with SCT, we found difficulties with emotion recognition and Theory of Mind. First, a difference was found for emotion recognition abilities between children with and without SCT, indicating deficits in young children with SCT (Chapter 5). The percentages of young children with SCT that score in the mild (8.5%) and severe clinical range (32.8%) are comparable with earlier research in older children and adolescents with SCT (Van Rijn, de Sonneville, & Swaab, 2018; Samango – Sprouse et al., 2018; van Rijn et al., 2014b). Similar, difficulties were found in overall Theory of Mind, indicating that young children with SCT have difficulties with understanding mental states of others. The proportion of young children with SCT that score in the mild (29.5%), and severe clinical range (6.6%) reveal that substantial Theory of Mind deficits already can be found early in development (Chapter 4). For both emotion recognition and Theory of Mind, we found that as the age of children cross-sectionally increases, the difference between children with SCT and their typically developing peers enlarges, with more difficulties in older children with SCT.

'Growing into deficit' phenomenon in early social cognitive functioning in SCT

The phenomenon that neurodevelopment becomes increasingly deviant from typically peers, as found in the present thesis for eye gaze to social important information, social orienting, emotion recognition and Theory of Mind (but not for joint attention) is often seen during the development of children with genetic syndromes and neurodevelopmental disorders. This concept is referred to as 'growing into deficit' (e.g. Rourke,1983; Sprong, 2008). Effects of genetic variations often become more apparent later on in development, when a child is faced with more complex developmental tasks and when compromised brain maturation leads to an increasing discrepancy with the age-required norms. From a neuropsychological perspective, the functionality of brain networks unfolds as a result of neuroanatomical maturation, which is reflected in increasing neurocognitive abilities and behavioral opportunities in the developing child. The development of neurocognitive functions occurs in a relative stepwise sequential pattern, in which the next step is dependent on the succession of previous steps. Early disturbances of neuroanatomical grow and the forming of interactive brain networks such as the 'social brain', for a substantial part drive by genetic factors such as SCT (Raznahan et al., 2016), could therefore impact the succession of an upcoming developmental step (Grossmann & Johnson, 2007). However, the impact of these disturbances may only emerge into cognitive difficulties later on in development when a developmental task is presented for which the brain is not fully equipped. Also, functional brain development involves the reorganization of interactions between different cortical structures and regions, based on a process of specialization of the brain into increasingly fine-tuned functions (Johnson, 2001; Johnson et al., 2005). For example, more complex aspects of social cognition, such as Theory of Mind, will be dependent on the maturation of associated cortical regions. This cortical maturation effect of interactive specialization, driven by genetic make-up, makes it possible that the impact of disturbances in early brain maturation may only become visible many years later in development.

On neurocognitive and behavioral level, impairments in fundamental social cognitive abilities early in life as found in the present thesis in young children with SCT may lead to a cascade of negative developmental effects. This negative loop of increasing difficulties may impact the development of social adaptive behavior during childhood, adolescence into adulthood, including the forming and maintaining of reciprocal

social relationships.

Longitudinal perspective: early social cognition predicting later ASD symptoms in SCT

Information on early developmental pathways in young children with SCT precursing social impairments later in life is extremely limited. Because of the well supported developmental continuity between early social cognition, in particular joint attention and social (cognitive) and communicative development (Franchini et al., 2019), we explored whether joint attention abilities in young children with SCT longitudinally predict severe social impairments, as reflected in ASD symptomatology.

The results of this study (Chapter 6) demonstrated that in young children with SCT, the tendency to coordinate attention between social partners (i.e. joint attention) is predictive of ASD symptoms at one year follow-up. Interestingly, we found differences in the two age groups with regard to the specific type of joint attention in predicting ASD symptoms. Whereas only responding to joint attention predicts ASD symptoms in 2-4 year old children with SCT. Both responding to and initiatings of joint attention predict ASD symptoms in children aged 4 years and older. The results of our study indicate that, while children with SCT grow up, challenges with the ability to initiate joint attention in which social motivation is involved (see for a review on neuroimaging studies that investigated brain networks supporting joint attention: Mundy, 2018), seems to be a pivotal marker in the emergence of severe social impairments and related risk for ASD symptoms, next to the ability to spontaneously follow joint attention bids of social partners that is important across both age groups.

Efficacy of a neurocognitive training program on early social cognition

Due to recent advances in noninvasive prenatal testing technology (i.e. the introduction of the NIPT; Samango-Sprouse et al., 2017; Tartaglia et al., 2020), it is possible to identify SCT as early as prenatally, resulting in increasing diagnoses of SCT. However, to date, there has been no research evaluating the potential effects of early and preventive neurocognitive training in SCT. Therefore, we aimed to

investigate the efficacy of a computer-based neurocognitive training program in 4-8 years old children with SCT, targeting at improving the understanding of social cues from facial expressions. Promising results regarding the effectiveness of the training were found, revealing that following the 4-week home based neurocognitive training was effective in significantly improving the ability to identify and match basic and complex facial expressions in children with SCT, with a large effect size (Chapter 7). These findings were irrespective of level of global cognitive functioning and expressive and receptive language abilities. After completing the training program, children with SCT showed emotion recognition abilities to a level that could not be distinguished from the typically developing group. These findings illustrate that there are opportunities for positively supporting the development of emotion understanding in children with SCT, already at a young age. Given the evidence that in SCT early social cognitive vulnerabilities may emerge and present more profoundly with age, early support of early social cognitive development may alter adverse developmental trajectories of young children with SCT, thereby mitigating the negative long-term impact of SCT on social adaptive functioning (Mundy & Neal, 2001; Dawson, 2008).

Differences between karyotype-subtypes of SCT (XXX, XXY, XYY)

It is important to consider evidence of the impact of SCT on social (cognitive) functioning in light of various factors that could contribute to research outcomes in genetic variations. We will evaluate three of these factors: the influence of specific karyotype-subtype and the role of time of diagnosis (prenatal, postnatal) and recruitment bias.

First of all, based on the convergent effects of the X and Y chromosomes on brain areas key to social cognition (Raznahan et al., 2016), and on earlier studies which showed no XXX/XXY differences in social behavior and social cognition (see for example: Van Rijn et al., 2014a; Van Rijn et al., 2014b), we believe that considering the SCT group as one study sample is appropriate and relevant. However, it should be noted that there are studies reporting more social behavioral impairments in the XYY-karyotype group (see for example: Tartaglia et al., 2017). Therefore, to account for karyotype-specific differences in social adaptive functioning, we also analyzed the impact of karyotype-subtype (XXX, XXY, XYY) on social behavioral and cognitive outcomes.

Overall, we found only minor differences between the three karyotype-subtypes on social outcomes, indicating strong similarities in social developmental vulnerabilities of children with XXX, XXY, and XYY. No differences between karyotype-subtypes were found for the domains of early behavioral social communication (Chapter 2), social interactions under varying levels of social load (Chapter 3), and for most measures of early social cognition (Chapter 4 and 5). These results support the findings of Raznahan et al. (2016), indicating that the X and Y chromosome exert congruent effects on brain systems involved in social adaptive functioning. However, relative to SCT groups having an extra X chromosome, we found a particularly pronounced vulnerability for social impairments in boys with XYY with regard to social orienting (Chapter 4), and reported ASD symptoms (Chapter 3). These findings suggest a more evident profile of social impairments in XYY with stronger association with a typical ASD behavioral profile, consistent with earlier studies that compared social impairments across SCT karyotypes. For example, it was found that school-age boys with XYY have a higher risk for clinical diagnoses of ASD, compared to boys and girls with an extra X chromosome (Cordeiro et al., 2012; Ross et al., 2012; Tartaglia et al., 2017; Wilson, King, & Bishop, 2019). However, in our study, when investigating ASD symptomatology in young children with SCT, we did not found a difference between karyotype-subtypes (Chapter 6). These findings suggest that besides the adverse impact of the X and Y chromosome on social adaptive functioning, and underlying brain systems, other biological factors may play a role in behavioral outcomes. It is for example hypothesized that the impact of the extra X or Y chromosome is amplified when genes that are expressed from these sex chromosomes interact with autosomal genetic variants that usually only have mild effects on behavioral outcomes ('double hit' hypothesis; Skuse, 2018). It would be interesting to further study developmental pathways of boys with 47,XYY in studies with larger sample sizes, also considering the scarce knowledge that is available for this group of children.

All in all, based on the majority of our data showing no karyotype specific differences, we consider the SCT group including the three different karyotypes (XXX, XXY, XYY) as an 'at risk' group, in which we can study developmental pathways of social behavior and social cognition. These results from the present thesis show that SCT is associated with vulnerabilities in social adaptive functioning, independent of karyotype-subtype (XXX, XXY, XYY), and that developmental factors may be more determinant of outcomes in the entire group of children with SCT, as compared to

karyotype-subtype. Although we cannot exclude that the relative risk per karyotype may slightly differ in some aspects, this risk also differs from individual to individual within this population.

Influence of time of diagnosis and recruitment bias on social adaptive functioning

Second, recruitment and ascertainment biases are always important factors in research in genetic variations, and could possible contribute to the variance in the phenotype of SCT, limiting the generalizability of research outcomes (Van Rijn, 2019). Therefore, it is important to distinguish recruitment cohorts in studies on SCT, for example cohorts recruited for research purposes versus clinical samples. Cohorts that are identified through prenatal screening may underestimate severity of the SCT phenotype, because of higher socioeconomic status leading to earlier (preventive) support and intervention, as for example speech and language support. On the other hand, study cohorts that are identified based on postnatal identification may likely overestimate severity of the phenotype, because of the selection of more severe cases. For that reason, it is impossible to study the full spectrum of strengths and weaknesses in children with SCT, when using only one recruitment strategy.

It is therefore crucial to take recruitment and ascertainment bias into account when considering the outcomes of studies in genetic variations such as SCT. In the present thesis, we evaluated the impact of these biases on outcomes of the studies, by describing the study samples in terms of prenatal or postnatal diagnosis. Our study samples consisted of a mix of prenatal and postnatal diagnosed children, and on average across the six studies, 72.4% of the children was diagnosed prenatally and 27.6% of the children postnatally, making it possible to investigate the developmental impact of SCT prospectively from an early age on, and suggesting that our findings are highly representative for the group of diagnosed children.

Furthermore, we investigated differences on study outcomes between the three recruitment cohorts (prospective follow-up after prenatal diagnosis; information seeking parents after prenatal or postnatal diagnosis; clinically referred cases after prenatal or postnatal diagnosis). We find no differences on social behavioral and social cognitive outcomes between these recruitment cohorts, except for one eyetracking

parameter (i.e. social attention to faces in the Dynamic Social Information paradigm, see Chapter 4). These results indicate that the findings of the present thesis are representative for the whole group of diagnosed children with SCT. Although in all cases the knowledge of having a child with SCT might influence the perspective on parenting behavior, these biases are mitigated by the use of objective measurements as used in the present thesis. However, as SCTs are also one of the most frequently underdiagnosed chromosomal conditions (Berglund et al., 2019), it remains unsure to what degree the findings can be generalized to those who have SCT, but remain undiagnosed.

The role of language and global cognitive functioning in social adaptive functioning

Although there is significant variability among individuals with SCT, the extra X or Y chromosome is not only associated with challenges in social adaptive functioning, but also with vulnerabilities related to social adaptive functioning such as on domains of emotional functioning, executive functioning, and the understanding and use of language (Kuiper et al., 2021; Urbanus et al., 2021; Van Rijn, 2019). It is therefore important to consider the challenges children with SCT face during social interactions in the context of broader development, as for example global cognitive, receptive and expressive language development. In the current thesis, we explore the role of global cognitive and language abilities in social adaptive development of young children with SCT. In all six studies, we found ethat level of receptive and expressive language and cognitive development could not explain the observed differences on social (cognitive) development between young children with SCT and their typically developing peers. The results also revealed that social impairments as reflected in symptomatology of ASD were found in both low IQ (IQ < 85) and average IQ groups (IQ > 85), indicating that young children with SCT show an equal amount of ASD symptoms across different levels of cognitive functioning. We can therefore conclude that SCT has a specific impact on early social behavioral and cognitive development, suggesting that social dysfunction later in life are anchored in a vulnerable social development in the first years of life.

Relative strengths in the social behavioral phenotype of children with SCT

Besides the impact that SCT appears to have on various aspects of social adaptive functioning and social cognitive mechanisms driving these social challenges, there are also areas of relative strength in the social behavioral phenotype of young children with SCT. Specifically, in the 12-24 month age group, we found intact abilities to initiate social interaction, tentatively suggesting that 12-24 month old children with SCT have a motivation to spontaneously seek and share affective experiences with others (Chapter 1). Also, on domains of social impairments in 3-8 year old children with SCT, social awareness is an area of relative strength, including abilities such as being aware of certain social cues as for example facial expressions and body language (Chapter 2). These results may suggest that children with SCT are relatively aware of their social environment, which may be different from children with ASD, a reasoning that has already been suggested by van Rijn et al. (2014a) and Wilson, King, & (2019). These findings are in line with the results of structured play observations in the whole age range of the sample from the present thesis (1-8 years), revealing that level of social input and demands from the environment indicate specific types of social deficits, and that vulnerabilities in social interaction behavior seems to be less visible when social load and thus social demand from the environment is low (Chapter 2). On the other hand, we did found an impact of SCT on social attention, indicating that children with SCT are less inclined to automatically and visually orient towards social relevant information. Together, these results may indicate that young children with SCT are aware of their social environment to a certain degree, but experience challenges in adequately directing their attention to social cues and in shaping their behavior during social interactions.

Earlier studies on parent-reports of social motivation of children with SCT from the age of four years found mixed results: depending on age groups and included karyotypes included in the study, no impact of SCT on social motivation was found in 4-18 years old with XXY or XYY by Cordeiro et al. (2012), whereas two other studies did find an impact of SCT on social motivation in 9-18 years old with XXX or XXY (van Rijn et al., 2014a) and 6-21 years old with XXY (Tartaglia et al., 2010).

With respect to awareness of the social environment, the existing evidence is more unambiguously, supporting the findings from the present thesis: Tartaglia et al.

(2010) found a relative strength in social awareness in boys with XXY, and Cordeiro et al. (2012) found mean scores for social awareness, just above the normal range cutoff in boys with XXY and XYY. Follow-up studies are needed to explore the developing pathways of social awareness and motivational mechanisms in young children with SCT from the first years of life into childhood. This is especially important in order to find targets of early and preventive support and intervention, based on the idea that early intact motivation for and awareness of social interactions should be preserved over the course of development.

Strengths, limitations and future directions

The present thesis is the first case-controlled study investigating the impact of SCT on the early social behavioral phenotype and underlying social cognitive developmental profiles, and has both strengths and limitations. Strengths include the relatively large, homogeneous, international sample of children, that consisted of children varying in time of diagnosis (pre- or postnatal) and recruitment strategy. With the large sample size of this study, we were able to not only investigate the early impact of SCT on social adaptive functioning and underlying social cognitive mechanisms, but also the age dynamics in specific age groups, giving insights in the developmental strengths and weaknesses in social (cognitive) mechanisms during early development.

Second, social adaption was investigated using objective and sensitive eyetracking techniques, in addition to structured behavior observations and parent reports in daily life settings, thereby investigating social adaption on a broad spectrum of outcomes. These sensitive measures of social adaptive behaviors allow us to study social development in children with SCT, independent of parent perspectives. Lastly, most outcomes in children with SCT in the present studies did not differ across international research sites (The Netherlands vs. United States), indicating a high degree of similarity in social cognitive functioning among children with SCT in Western cultures, and consequently proving the robustness of the results.

Several important side notes need to be taken into consideration when interpreting the outcomes of the present thesis. Limitations include the cross-sectional design of most of the studies presented in this thesis, that limits cause-effect conclusions. Our interpretation of age dynamic effects is based on comparison of age cohorts rather than

longitudinal assessment, although we did investigate the longitudinal and predictive value of joint attention on later ASD symptoms in SCT. Future studies should further focus on the longitudinal development of social adaptive functioning in children with SCT, which will be explored in this population of children with SCT with prospective follow-up. With the help of longitudinal studies it is possible to investigate whether markers in early development, such as for example joint attention but also other factors in domains of executive functioning and/or language are predictive of later vulnerable outcomes and clinical diagnoses of neurodevelopmental disorders, such as ASD and Attention Deficit/Hyperactive Disorder (ADHD).

Second, the majority of individuals with SCT still remains undetected during life (Berglund et al., 2019), although the group of diagnosed young children is rapidly growing with the introduction of the NIPT that makes it possible to diagnose SCT even before birth, dependent on the political standards in the specific country. In the present thesis, social outcomes were mainly not dependent on recruitment strategy (i.e. prospective follow-up group, information seeking parents group, or clinically referred cases group), which suggests that our findings are representative for this group of diagnosed children. However, it remains unsure to what degree the findings in this study can be generalized to those who have SCT, but remain undiagnosed.

Third, we included boys with 47,XXY (Klinefelter Syndrome) in our study regardless whether children had received testosterone treatment. To our knowledge, one randomized controlled trial (RCT) assessed outcomes of androgen treatment on parent reported social behavioral outcomes in children, aged 4-12 years (Ross et al., 2017). In this RCT, improvements after 24 months of androgen treatment were found in parent reported social problems of their child. Although a percentage of boys with 47,XXY (Klinefelter Syndrome) received testosterone treatment (46%) in the present study, the sample is not powered to study the efficacy of testosterone treatment on social development. Randomized and placebo-controlled trials should therefore further investigate the effects of testosterone treatment on social (cognitive) outcomes in young children with Klinefelter syndrome (one is currently underway: PI Davis, NCT03325647).

Fourth, we were not able to investigate background family history and early environmental experiences that may shape social adaptive (cognitive) development

in children with SCT. The variability in phenotypes of individuals of SCT provides an unique opportunity to investigate not only genetic but also environmental aspects that contribute to neurodevelopmental outcome. Early environmental factors could possible moderate neurodevelopmental outcomes in children with SCT, calling for a more integrative perspective considering vulnerabilities in developmental in children with SCT in the context of environmental experiences. To our knowledge, one study investigated early stressful life experiences in relation to social cognitive outcomes in children with SCT (Van Rijn et al., 2018). The results of this study show that 9-18 year old children with an extra X chromosome are vulnerable to adverse early life events, with social cognition being particularly impacted. These findings support the hypothesis that early life stress may impact brain development upon the development of brain areas that are already more vulnerable in individuals with SCT (Raznahan et al.,2016). More insight in early environmental aspects that shape social cognitive developmental outcomes in young children with SCT, may help to identify prospective factors that could be targeted to minimize neurodevelopmental impact.

Finally, in the present thesis, we particularly focused on the impact of SCT on social adaptive functioning. The results from recent reviews from our research group (Van Rijn, 2019; Urbanus, Van Rijn & Swaab, 2019) suggest that besides social cognition, the domains of language, executive function and emotion regulation are domains of developmental vulnerability that may be key factors underlying risk for neurobehavioral problems. Knowledge on the early impact of SCT on domains of language and executive function is currently growing (see for example Kuiper et al., 2021; Urbanus et al., 2021; Capelli et al., 2022). As symptoms of psychopathology are caused by a complex interplay of neurocognitive strengths and weaknesses, it may be interesting to identify subgroups of children with SCT that share a specific neurocognitive profile on domains of language, social cognition, executive functioning and emotion regulation, underlying social impairments and related psychopathology. Since children with SCT show impairments across multiple neurocognitive domains, insights in the interaction between neurocognitive features may provide more information about developmental pathways shared by a subgroup of children with SCT, rather than assessing single neurocognitive risk factors (Borsboom & Cramer, 2013).

Despite those limitations we still feel confident to conclude that social behavioral

functioning and underlying social cognitive mechanisms are vulnerable in early development of young children with SCT, which leads to several implications for clinical practice.

Implications for clinical practice

The outcomes of the studies described in the current thesis reveal an impact of SCT on social adaptive development already from an early age on, suggesting an impact of the extra X or Y chromosome on neurodevelopment already in early childhood. The results reveal a relatively stable impact of SCT on social behavioral level and related ASD symptoms at an early age and across different karyotypes (XXX, XXY, XYY). However, on social cognitive level, age related effects are found, with more challenges in acquiring age-related social cognitive milestones in older age groups. Joint attention, an important social cognitive milestones in typical development, is an exception to this rule, as we found that Joint attention difficulties are stable across the 1-8 years age span in children with SCT, and predictive of ASD symptoms over time. These insights may have considerable implications for clinical care.

The findings of this thesis underline the importance of incorporating closely monitoring of social adaptive behavioral and cognitive functioning in children with SCT as routine care within the first years of life. As we found that joint attention abilities are predictive for severe social behavioral impairments in terms of ASD symptoms, difficulties with joint attention can serve as an early marker for an 'at risk' developmental profile. Early detection and support of children at risk is relevant for several reasons.

First, parents of children with known SCT often experience stress and uncertainty about their child's development and their own parenting (Richardson, Riggan, & Allyse, 2021). Early detection of developmental risk in children allows for appropriate support for parents by offering psychoeducation and/or coaching. Early support of parents might result in higher parent well-being and family adaptive functioning by means of improved positive psychological functioning and the learning of effective coping strategies (see for examples about the efficacy of early parental support in the ASD literature: Estes, Swain, & MacDuffie, 2019; (Saccà, Cavallini, & Cavallini, 2019).

Second, children at risk for social impairments may have fewer social learning experiences compared to their peers on top of their social vulnerabilities, possibly leading to a cascade of negative developmental effects. For example, reduced attention to socially meaningful and complex stimuli already early in development may have substantial impact on the fundamentals of social learning. Reduced social attention may lead to limited quantitative and qualitative opportunities to acquire social knowledge in children with SCT, and to learn from (complex) social interactions (Mundy & Neal, 2001). Attending to another person's face and eyes allows typically developing children to have rich social experiences that are crucial for the development of social and communicative abilities, such as joint attention, language acquisition, and face or facial affect recognition (Gliga & Csibra, 2007). Consequently, less attention to the eyes and faces of others may have a broad impact on the complex maturation of social (cognitive) abilities, which are built upon basic social-perceptual information. It is therefore important to closely monitor patterns of social communication in young children from an early age on, as difficulties in early social communication (e.g. responding to joint attention, making eye contact) may be key indicators of compromised cross-domain development. Close monitoring of vulnerable children with SCT and if necessary early support and tailored intervention may positively influence social development through childhood.

Negative developmental cascade effects could explain why some vulnerabilities in early childhood predict widespread cross-domain difficulties or even impairments throughout childhood, adolescence and into adulthood. Given the effects that may spread over time on social adaptive domain, targeted interventions delivered at key windows of opportunity during development could interrupt negative or promote positive cascades (Masten & Cicchetti, 2010). Preventive support of social abilities and treatment early in life of children with SCT is therefore essential. In the present thesis, a promising effect of a neurocognitive training targeting facial emotion recognition, was found (see Chapter 7). The results of this study show that attending a 4-week home based training was effective in significantly improving the ability to identify and match basic and complex facial expressions in 4-8 year old children with SCT. These findings illustrate that there are opportunities for positively supporting social cognitive development in children with SCT, already at a young age. As neurocognitive training programs are easy accessible and cost-effective, support of early neurocognitive development can be preventively used in home-based and

school-based settings. Neurocognitive training programs can also be used as part of a tailored and integrative intervention program for young children with SCT at risk of specific social cognitive vulnerabilities, which has become visible based on individual assessment of neurocognitive strengths and weaknesses. Early support of social cognitive development may alter adverse developmental trajectories of young children with SCT, and minimize the negative long-term impact of SCT on social adaptive outcomes (Mundy & Neal, 2001; Dawson, 2008).

Third, as early behavioral therapeutic interventions are well established to support early development of children with idiopathic ASD and their families (see for a review: French & Kennedy, 2018), these early services can possibly be beneficial for young children with SCT as well (Tartaglia et al., 2017). With regard to these early behavioral interventions, a number of treatment programs have shown to be beneficial for young children with ASD or at risk for ASD, as for example Applied Behavioral Analysis (ABA), Early Start Denver Model, Floortime, Pivotal Response Treatment (PRT), and Verbal Behavior Therapy (Bishop-Fitzpatrick, Minshew, & Eack, 2014; (Cadogan & McCrimmon, 2015; French & Kennedy, 2018; (Pajareya, Sutchritpongsa, & Kongkasuwan, 2019). It is of urgent need to further investigate the possible efficacy and long-term outcomes of these services and programs early in life of children with SCT, even more considering the need to offer supportive preventive interventions to the growing group of (prenatally) diagnosed children with SCT (Samango-Sprouse et al., 2017; Tartaglia et al., 2020).

Last, as the rates of SCT diagnoses in infants and children are rapidly increasing, there is a need for specialized interdisciplinary care to address associated risk. Although there is significant variability among individuals with SCT, the extra X or Ychromosome is not only associated with challenges in social adaptive functioning, but also with vulnerabilities related to social adapttive functioning such as emotional functioning, cognitive functioning, executive functioning, understanding and use of language, adaptive/daily living skills. Besides, medical conditions such as hypogonadism in 47,XXY/Klinefelter Syndrome could influence social position of children with 47,XXY (Tartaglia et al., 2015; van Rijn, 2019). Currently, children and adolescents with SCT often receive care by genetic clinics where families are provided with information about the genetic condition after (prenatal) diagnosis and by pediatric endocrinologists that have experience with androgen treatment

in XXY (Gravholt, Tartaglia, & Disteche, 2020). However, in addition to genetic and endocrinology care, neurodevelopmental issues need to be addressed, more specific early social adaptive (cognitive) functions. Comprehensive and coordinated multidisciplinary care is important to provide specialized assessment and treament in children and adolescent with SCT, such as the Dutch TRIXY Center of Expertise (www.trixyexpertisecentrum.nl; Leiden, The Netherlands). Evidence-based international consensus or guidelines are crucial to provide best quality care for individuals with SCT and their families, also in other parts of the world.

Conclusion

In conclusion, the results presented in the current thesis reveal that already very early in development, SCT is associated with vulnerabilities in social behavioral functioning and underlying early social cognitive mechanisms. It is found that SCT impacts social behavioral development from the first years of life, reflected in vulnerabilities in early social communication and social withdrawal during social interactions. Early social cognitive dysfunctions that may underlie social adaptive vulnerabilities in SCT include social orienting, joint attention and more complex social cognitive abilities such as the understanding of facial emotions and Theory of Mind. These results suggest a specific profile of social (cognitive) vulnerabilities in young children with SCT, informing close evidence-based early monitoring and targeted support when necessary. Also, it became clear that there are opportunities for positively supporting development of social cognition, specially facial emotion recognition, in young children with SCT.

These findings may signify at risk early development in children with SCT, and may help explain reported social vulnerabilities and related risk for psychopathology later on in life. Challenges in social development and difficulties with social adaptive functioning highly impact quality of life (de Vries & Geurts, 2015). Therefore, the findings of the present thesis advocate for close monitoring from the earliest stages of social behavioral and cognitive development throughout early childhood and risk on social impairments related to ASD. Early implementation of (preventive) support and intervention has the potential to minimize neurodevelopmental impact of the extra X or Y chromosome thereby optimizing quality of life of children with SCT and their families.

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NEDERLANDSE SAMENVATTING

Nederlandse samenvatting en discussie

In het huidige proefschrift wordt de impact van een extra X- of Y- chromosoom (47,XXX; 47,XXY; 47,XYY) beschreven op vroeg sociaal adaptief gedrag en de sociaal-cognitieve ontwikkeling bij kinderen van 1 tot 7 jaar. De resultaten van de studies gerapporteerd in dit proefschrift maken deel uit van de TRIXY Studie bij jonge kinderen (Universiteit Leiden, Nederland), één van de eerste internationale onderzoeken waarin vroege neurocognitieve en gedragsmatige uitkomsten in de eerste levensjaren van kinderen met een extra X- of Y-chromosoom prospectief in kaart worden gebracht. In deze Nederlandse samenvatting en discussie worden na een algemene introductie op het onderwerp de onderzoeksvragen en het belang van het onderzoek naar deze aspecten van de ontwikkeling beschreven, waarna de belangrijkste bevindingen van de zes studies worden samengevat en bediscussieerd. Vervolgens worden de sterke punten en de beperkingen van de uitgevoerde studies beschreven en worden tenslotte de implicaties benoemd voor de klinische zorg voor individuen met een extra X- of Y-chromosoom.

Introductie: een extra X- of Y- chromosoom (XXX, XXY, XYY)

Bij een trisomie van de geslachtschromosomen is er sprake van een extra X- of Y-chromosoom in tegenstelling tot het typische karyotype van 46,XX bij meisjes en 46,XY bij jongens. Deze trisomieën worden veroorzaakt door een spontane deelfout tijdens de vroege celdeling (Leggett, Jacobs, Nation, Scerif, & Bishop, 2010). De schattingen van de prevalentie van deze X- en Y-gebonden trisomieën variëren van 1:650 tot 1:1000 en deze trisomieën zijn dus niet zeldzaam (Boyd, Loane, Garne, Khoshnood, & Dolk, 2011). Er is een grote variabiliteit in het fenotype bij individuen met een extra X- of Y- chromosoom. Het mild fysieke fenotype kenmerkt zich door minimale atypische gezichtskenmerken, een lange gestalte en een lage spierspanning. Een extra X- of Y-chromosoom geeft ook een verhoogd risico op somatische, neurologische, neurocognitieve, gedrags- en psychologische kwetsbaarheden tijdens de ontwikkeling en in het volwassen leven (Tartaglia et al., 2015). De belangrijkste neurocognitieve domeinen van kwetsbaarheid in de kindertijd en adolescentie zijn de taalontwikkeling, het sociaal adaptief functioneren en het executief functioneren. Het globaal cognitief functioneren binnen de groep individuen met een extra X- of Y-chromosoom is variabel, variërend van een laag gemiddeld tot boven gemiddeld IQ; meestal ligt het cognitief functioneren in de laag gemiddelde tot gemiddelde

range (Urbanus, Van Rijn, & Swaab, 2020).

Onderzoek bij volwassen, adolescenten en schoolgaande kinderen hebben laten zien dat sociaal adaptief functioneren één van de belangrijkste domeinen van kwetsbaarheid is bij personen met een extra X- of Y-chromosoom (Van Rijn, Urbanus, & Swaab, 2019; Urbanus, Van Rijn, & Swaab, 2020; Tartaglia et al., 2020). Een extra X- of Y-chromosoom uit zich op gedragsniveau bijvoorbeeld door verlegenheid, uitdagingen bij het vormen van interpersoonlijke relaties, een verhoogde mate van sociale angst en sociale impulsiviteit, naast stoornissen in onderliggende sociaal-cognitieve mechanismen (zie voor reviews: Freilinger et al., 2018; Tartaglia, Hoowell, Sutherland, Wilson, & Wilson, 2010; Ross et al., 2012; Urbanus, Van Rijn, & Swaab, 2020). De ernst van deze sociale kwetsbaarheden wordt geïllustreerd door een verhoogd risico op symptomen van Autisme Spectrum Stoornissen (ASS; Van Rijn, 2019). Het is echter nog niet bekend wat de invloed is van het extra X- of Y-chromosoom op de sociale ontwikkeling in de eerste jaren van het leven.

De relevantie van het bestuderen van genetische variaties

Genetische variaties zoals trisomieën van de X- en Y-chromosomen, met specifieke uitkomsten in neurocognitief en gedragsmatig functioneren, kunnen ons helpen om ontwikkelingsrisico's te begrijpen die ten grondslag liggen aan complexe gedragsfenotypes en psychopathologie. Op die manier dienen trisomieën van de X- en Y-chromosomen als een uniek risicomodel van de sociale ontwikkeling en bieden ze de mogelijkheid om meer inzicht te krijgen in ontwikkelingspaden die moeilijker te bestuderen zijn in populaties die gedefinieerd zijn op basis van een gedragsfenotype (Reiss, Eliez, Schmitt, Patwardhan, & Haberecht, 2000). Een extra X- of Y-chromosoom kan al prenataal worden vastgesteld in tegenstelling tot gedragsgedefinieerde classificaties zoals een Autisme Spectrum Stoornis (ASS). Dat zorgt ervoor dat vroege ontwikkelingspaden prospectief en al vanaf het eerste levensjaar in beeld gebracht kunnen worden. De vroege identificatie van een kwetsbare sociale ontwikkeling kan helpen om ontwikkelingspaden naar sociale stoornissen en psychopathologie in kaart te brengen, wat zicht geeft op ontwikkelingsmechanismen in bredere zin, zoals op vroege markers van een risicovolle ontwikkeling. Deze inzichten zijn niet alleen nuttig voor de populatie individuen met een extra X- of Y-chromosoom, maar ook voor populaties die pas

op latere leeftijd kunnen worden gediagnosticeerd op basis van gedragskenmerken en symptomen, zoals populaties met neurobiologische ontwikkelingsstoornissen. De studies gepresenteerd in het huidige proefschrift beogen te voorzien in het verkrijgen van inzicht in de vroege ontwikkelingspaden van ontwikkelingsrisico. We hebben ons daarbij gericht op domeinen waarvan uit eerder onderzoek bekend is dat deze kwetsbaar zijn in individuen met een extra X- of Y-chromosoom, namelijk sociaal gedrag en onderliggende sociaal cognitieve mechanismen. De overkoepelende vraag is of al op zeer jonge leeftijd factoren in de ontwikkeling geïdentificeerd kunnen worden die wijzen op een kwetsbaarheid in de sociale ontwikkeling.

Doel, onderzoeksvragen en design

Het doel van het huidige proefschrift is het onderzoeken van de impact van een extra X- of Y- chromosoom (47,XXX; 47,XXY; 47,XYY) op vroeg sociaal adaptief gedrag en de sociaal-cognitieve ontwikkeling bij kinderen van 1 tot 7 jaar. De volgende onderzoeksvragen staan in het proefschrift centraal. Als eerste, gezien de literatuur sociaal adaptieve problemen beschrijft bij volwassenen, adolescenten en schoolgaande kinderen met een extra X- of Y-chromosoom, hebben we ons de vraag gesteld wat de impact is van een extra X- of Y-chromosoom op vroeg sociaal adaptief gedrag. Ten tweede, om zicht te krijgen op onderliggende oorzaken van deze impact op sociaal adaptief gedrag, hebben we ook gekeken naar de neurocognitieve bouwstenen van gedrag. We hebben daarom de vraag beantwoord hoe een extra X- of Y-chromosoom van invloed is op de ontwikkeling van vroege sociaal-cognitieve vaardigheden (visuele oriëntatie op sociale informatie, joint attention, emotieherkenning en Theory of Mind). Ten derde onderzochten we de ernst van sociale kwetsbaarheden bij jonge kinderen met een extra X- of Y-chromosoom, door te onderzoeken of een extra X- of Y-chromosoom geassocieerd is met het optreden van symptomen die typisch zijn voor ASS. We vroegen ons daarbij af of deze klinisch relevante sociale kwetsbaarheden te voorspellen zijn en onderzochten daarom hoe een vroege sociaal-cognitieve marker (joint attention) bij jonge kinderen met een extra X- of Y-chromosoom longitudinaal gerelateerd is aan sociale beperkingen op latere leeftijd, zoals gereflecteerd in symptomen van ASS. Tot slot, aangezien de literatuur over het (preventief) ondersteunen van de sociale ontwikkeling van kinderen met een extra X- of Y-chromosoom zeer beperkt is, hebben we ons gericht op de vraag of een neurocognitief trainingsprogramma mogelijk effectief is om een kern onderdeel

van sociale cognitie te beïnvloeden, namelijk het begrijpen van sociale signalen in gezichtsuitdrukkingen. We hebben de werkzaamheid van dit programma onderzocht bij kinderen van 4-8 jaar met een extra X- of Y-chromosoom.

We bestudeerden meer dan 100 kinderen met een extra X- of Y-chromosoom in de leeftijd van 1 tot 7 jaar die we vergeleken met een groep van 100 typische ontwikkelingsgroep met dezelfde leeftijd. Werving van de participanten en dataverzameling vonden plaats op twee locaties: het TRIXY Expertisecentrum van het Leids Universitair Behandel en Expertise Centrum (LUBEC) van de Universiteit Leiden in Leiden (Nederland) en de eXtraordinary Kids Clinic van het Children's Hospital Colorado in Denver (VS).

Samenvatting van de belangrijkste bevindingen

De belangrijkste bevindingen van de zes studies die worden gepresenteerd in het huidige proefschrift kunnen als volgt worden samengevat:

1. Een extra X- of Y-chromosoom heeft invloed op de sociaal adaptieve gedragsontwikkeling vanaf de eerste levensjaren, weerspiegeld in kwetsbaarheden in vroege sociale communicatie en sociale interactie. In een gedragsobservatie paradigma waarbij een volwassene op een gestructureerde wijze sociale interacties aangaat met het kind, werd duidelijk dat kinderen met een extra X- of Y-chromosoom minder vaak oogcontact maken, minder non-verbale communicatie inzetten en meer sociale teruggetrokkenheid laten zien. Dit is met name het geval in situaties waarin de volwassene meer actief sociaal interacteerde en kinderen dus meer sociale prikkels moeten verwerken (Hoofdstuk 2 en 3).

2. Vroege sociaal cognitieve disfuncties zijn al op jonge leeftijd waarneembaar bij kinderen met een extra X- of Y-chromosoom. Met behulp van eyetracking methoden en neurocognitieve testen vonden we dat kinderen met een extra X- of Y-chromosoom verschillen laten zien met hun leeftijdsgenoten als het gaat om visuele oriëntatie op sociale signalen, joint attention, emotieherkenning en Theory of Mind. Deze bevindingen suggereren dat sociaal cognitieve kwetsbaarheden die in de literatuur beschreven zijn bij schoolgaande kinderen,

adolescenten en volwassenen met een extra X- of Y-chromosoom al zichtbaar zijn in kwetsbaarheden in de eerste jaren van het leven (Hoofdstuk 4 en 5).

3. De vroege sociaal cognitieve ontwikkeling van kinderen met een extra X- of Y-chromosoom wordt gekenmerkt door een fenomeen van 'growing into deficit' (Hoofdstuk 4 en 5), zoals zichtbaar in de kwetsbare ontwikkeling van emotieherkenningsvaardigheden en Theory of Mind.

4. Met behulp van vragenlijsten en klinische interviews onderzochten we de ernst van de sociale kwetsbaarheden bij kinderen met een extra X- of Y-chromosoom, zoals weerspiegeld in symptomen van ASS. We vonden dat kinderen met een extra X- of Y-chromosoom een verhoogd risico hebben op symptomatologie die is geassocieerd met ASS, waarbij 22% van de kinderen boven de klinische cutoff scoort op zowel problemen in de sociale interactie en communicatie als beperkte interesses en repetitief gedrag (Hoofdstuk 3 en 6). Opvallend was een extra X- of Y-chromosoom met name impact heeft op het sociaal communicatieve domein, in vergelijking met de andere dimensie van ASS.

5. De geringe neiging tot joint attention is gerelateerd aan symptomen van ASS bij een follow-up van één jaar, en is daarom een vroege marker voor kinderen met een extra X- of Y-chromosoom die risico lopen op ernstige sociale beperkingen (Hoofdstuk 6). We vonden dat terwijl reageren op een uitnodiging tot joint attention (responding to joint attention) ASS-symptomen voorspelt bij 2-4 jaar oude kinderen met SCT, het reageren op joint attention (responding to joint attention) en het initiëren van joint attention (initiating joint attention) ASS-symptomen voorspellen bij kinderen van 4 jaar en ouder.

6. Een neurocognitieve training van emotieherkenningsvaardigheden heeft het potentieel om de neurocognitieve impact van het extra X- of Y-chromosoom op vroege sociaal cognitieve vaardigheden te verminderen (Hoofdstuk 7). We vonden dat na een 4-weekse intensieve en ouder-gemedieerde training gericht op het ondersteunen van de ontwikkeling van emotieherkenning, kinderen met een extra X- of Y-chromosoom verbeterden in hun vaardigheden om emoties te herkennen op gezichten. Na voltooiing van het trainingsprogramma presteerden deze kinderen vergelijkbaar met hun leeftijdsgenoten zonder extra

X- of Y-chromosoom die de training niet hadden gevolgd.

Discussie van de bevindingen

Vroeg sociaal en communicatief gedrag bij kinderen met een extra X- of Y-chromosoom

In het huidige proefschrift is beschreven dat kinderen met een extra X- of Y-chromosoom al zeer vroeg in de ontwikkeling, namelijk op de leeftijd tussen 12 en 24 maanden, kwetsbaarheden hebben in het vermogen om te communiceren met anderen en in dagelijks sociaal emotioneel functioneren (Hoofdstuk 2). We onderzochten het sociaal en communicatief gedrag bij kinderen met een extra X- of Y-chromosoom van 12 tot 24 maanden op drie aspecten: communicatief functioneren in een sociale interactie, de frequentie van oogcontact en sociaal emotioneel functioneren in het dagelijks leven. We gebruikten systematische gedragsobservaties om sociaal communicatief gedrag te onderzoeken wanneer kinderen daadwerkelijk worden blootgesteld aan sociale interacties in een gestructureerde spelsituatie. De bevindingen van deze gestructureerde spelobservaties lieten zien dat kinderen met een extra X- of Y-chromosoom van 12 tot 24 maanden gemiddeld een lagere frequentie van oogcontact vertonen bij het reageren op sociale communicatie dan hun leeftijdsgenoten. Verder gaven de resultaten aan dat deze kinderen problemen hebben met vroege sociale communicatie, namelijk met joint attention, het non-verbale uiten van verzoeken en met wederzijdse sociale interacties. De resultaten laten ook zien dat deze kwetsbaarheden in de sociale communicatie ook zichtbaar zijn in het sociaal emotioneel functioneren in het dagelijks leven. We vonden namelijk dat kinderen met een extra X- of Y-chromosoom gemiddeld meer moeite hebben met het vertonen van typisch sociaal-emotioneel gedrag, zoals het imiteren van fantasiespel en het wederzijds uitwisselen van emoties.

Vanuit ontwikkelingsperspectief is het bekend dat kwetsbaarheden in de vroege sociale communicatie en emotioneel functioneren een vroeg signaal zijn van risicovolle sociale ontwikkeling op de verdere sociale ontwikkeling (Mundy & Newell, 2007). Naast de impact van een extra X- of Y- chromosoom tijdens het tweede levensjaar van kinderen (zoals gevonden in Hoofdstuk 2), vonden we inderdaad ook een impact van het extra X- of Y-chromosoom op sociaal adaptief functioneren bij oudere leeftijdsgroepen (Hoofdstuk 3). Het met de leeftijd vergroten van het

vermogen tot sociale interactie is nodig om de vaardigheden te ontwikkelen om met complexere sociale informatie om te gaan (Soto-Icaza, Aboitiz, & Billeke, 2015). We waren daarom geïnteresseerd of en hoe een extra X- of Y-chromosoom van invloed is op de manier waarop kinderen in de leeftijd van 1 tot 7 jaar sociaal interactiegedrag vormgeven onder verschillende condities van sociale input in een gestructureerde spelsituatie. De resultaten van deze gestructureerde spelobservaties lieten zien dat kinderen met een extra X- of Y-chromosoom minder sociale betrokkenheid vertonen. De kinderen lieten namelijk minder interactief gedrag zien en meer onafhankelijk spel in vergelijking met leeftijdsgenoten. Interessant is dat verminderde sociale interactie en teruggetrokken gedrag verschilden als een functie van sociale input. Jonge kinderen met een extra X- of Y-chromosoom vertoonden in vergelijking met hun leeftijdsgenoten minder sociale interacties wanneer de sociale input afwezig was, en meer sociale terugtrekking wanneer de sociale input hoog was. De sociale beperkingen waren het meest uitgesproken wanneer het niveau van sociale input hoog was. Deze bevindingen laten zien dat sociale input en de eisen van de omgeving voorwaardelijk zijn voor de vorming van sociaal adaptief gedrag. De kwetsbaarheden in sociaal adaptief gedrag zoals beschreven in Hoofdstuk 3 bleken onafhankelijk te zijn van leeftijd, wat wijst op een stabiele impact van een extra X- of Y-chromosoom op sociaal gedragsmatig functioneren tijdens de vroege ontwikkeling.

De resultaten van Hoofdstuk 2 en Hoofdstuk 3 passen bij eerdere studies die de impact van het extra X- en Y-chromosoom op sociale fenotypes onderzochten in populaties met een hogere leeftijd en met brede leeftijdsranges. Deze studies toonden namelijk aan dat een extra X- of Y-chromosoom wordt geassocieerd met verlegenheid, sociale terugtrekking, problemen in relaties met leeftijdsgenoten, verminderde sociale assertiviteit en communicatieproblemen (Bender, Harmon, Linden, Bucher-Bartelson, & Robinson, 1999; Geschwind & Dykens, 2004; Otter, Schrander-Stumpel, & Curfs, 2010; Van Rijn et al., 2014a) en een verhoogd risico op milde symptomen van sociale angst (Van Rijn et al., 2014; Wilson, King, & Bishop, 2019). De bevindingen uit onze studies (Hoofdstuk 2 en 3) dragen bij aan de reeds bestaande kennis door aan te tonen dat een impact van een extra X- of Y-chromosoom op de sociaal adaptieve ontwikkeling al kan worden gevonden tijdens de eerste levensjaren, en dat deze impact groter is wanneer de complexiteit van sociale informatie hoger is.

Een extra X- of Y-chromosoom en symptomen van Autisme Spectrum Stoornis (ASS)

De ernst van kwetsbaarheden in sociaal gedrag bij schoolgaande kinderen, jongeren en volwassenen met een extra X- of Y-chromosoom wordt in de reeds bestaande literatuur geïllustreerd door een verhoogd niveau van symptomen en klinische diagnoses van ASS (zie voor een review: Van Rijn, 2019). Eerdere studies die de impact van een extra X- of Y-chromosoom op ASS-symptomatologie hebben vastgesteld, waren gericht op populaties met brede leeftijdsranges, waaronder individuen in de basisschool leeftijd tot in de volwassenheid. Vergeleken met een wereldwijde prevalentie van ASS van 0,6% in de algemene bevolking (Elsabbagh et al., 2012), zijn er aanwijzingen dat de prevalentie ASS hoger is bij individuen met een extra X- of Y-chromosoom, namelijk 15-30% (Van Rijn, 2019).

Het risico op ASS-symptomen bij genetische variaties zoals een extra X- of Y-chromosoom kan worden gemeten door de mogelijke impact van het extra X- of Y-chromosoom op een reeks sociale gedragsvaardigheden in kaart te brengen die normaal verdeeld zijn in de algemene bevolking (Constantino & Gruber, 2012). Een alternatieve benadering is om te onderzoeken of jonge kinderen met een extra X- of Y-chromosoom klinische symptomen van ASS laten zien. In dit proefschrift hebben we beide benaderingen gebruikt om de mogelijke impact van een extra X- of Y-chromosoom op risico van sociale beperkingen te bestuderen die geassocieerd zijn met ASS.

Bij de eerste benadering bestudeerden we de impact van het extra X- of Y-chromosoom op een spectrum van sociale gedragsvaardigheden (Hoofdstuk 3). We vonden minder sterke gedragsvaardigheden op alle gemeten dimensies: sociaal bewustzijn, sociale cognitie, sociale communicatie en sociale motivatie; jonge kinderen met een extra X- of Y-chromosoom vertoonden daarnaast ook minder brede interesses en minder variatie in gedrag.

De tweede benadering liet zien dat ASS-symptomen aanzienlijk vaker voorkwamen bij kinderen met een extra X-of Y-chromosoom in vergelijking met de algemene bevolking. 22% van de kinderen voldeed aan de classificatie criteria van ASS, op basis van de aanwezige symptomen (Hoofdstuk 6). Onze resultaten wijzen op een specifiek profiel van ASS-symptomen bij jonge kinderen met een extra X- of Y-chromosoom.

De resultaten gaven namelijk aan dat een extra X- of Y-chromosoom vooral de vaardigheden in het sociale en communicatieve domein beïnvloedt.

Samengevat impliceren de resultaten uit deze twee hoofdstukken (Hoofdstuk 3 en 6) een vroege impact van het extra X- of Y-chromosoom, leidend tot het een hoog risico op ernstige sociale beperkingen, zoals weerspiegeld in ASS-symptomatologie. De gevonden sociale beperkingen zijn van invloed op de interpersoonlijke communicatie en de ontwikkeling en instandhouding van adequate relaties met anderen (Rao, Beidel, & Murray, 2008). Het is daarom van belang te onderzoeken welke ontwikkelingsmechanismen betrokken zijn bij het optreden van deze pathologische symptomen. We onderzochten daarom vervolgens de impact van een extra X- of Y-chromosoom op sociaal cognitieve vaardigheden die in de typische ontwikkeling rijpen in de vroege kindertijd.

Cognitieve mechanismen van sociaal gedrag: de impact van een extra X- of Y-chromosoom op vroege sociale cognitie

Om de mechanismen te begrijpen die ten grondslag liggen aan de kwetsbaarheden in sociaal adaptief gedrag, is het van belang om te overwegen dat de aanwezigheid van een extra X- of Y-chromosoom invloed heeft op de rijping van hersenfuncties en -netwerken die betrokken zijn bij sociaal adaptieve cognitieve en gedragsontwikkeling, vaak aangeduid als het 'sociale brein' (Hong & Reiss, 2014; Raznahan et al., 2016). Sociale situaties bieden grote hoeveelheden informatie die snel en gelijktijdig moeten worden verwerkt. Deze situaties activeren sociaal-cognitieve mechanismen die belangrijke informatie selecteren om vervolgens deze informatie te verwerken en zodoende adequaat te kunnen reageren. Sociale cognitie wordt gedefinieerd als de cognitieve informatieverwerkingsmechanismen die ons in staat stellen om sociale informatie waar te nemen, te begrijpen en vervolgens te interacteren met de sociale omgeving (Beauchamp & Anderson, 2010). Sociaal cognitieve mechanismen staan centraal in de interpersoonlijke communicatie, in het ontwikkelen en onderhouden van relaties met anderen (Rao, Beidel, & Murray, 2008) en zijn geassocieerd met ervaren kwaliteit van leven (de Vries & Geurts, 2015). Aangezien de sociaal-cognitieve ontwikkeling verankerd is in de vroege hersenrijping en omdat de eerste levensjaren belangrijk zijn voor snelle rijping en specialisatie van het sociale brein, zijn de eerste levensjaren een belangrijke periode voor het behalen

van mijlpijlen in de sociaal cognitieve ontwikkeling, waardoor succesvolle sociale interacties met anderen mogelijk gemaakt kunnen worden (Grossmann & Johnson, 2007). Deze vroege sociaal cognitieve functies hebben in de typische ontwikkeling verschillende ontwikkelingstrajecten en ontvouwen zich in verschillende stadia van de ontwikkeling, afhankelijk van de rijping en specialisatie van breingebieden die betrokken zijn bij sociaal cognitieve functies (Soto-Icaza, Aboitiz, & Billeke, 2015). Om zicht te krijgen op sociaal cognitieve mechanismen die ten grondslag liggen aan verminderd sociaal adaptief gedrag bij kinderen met een extra X- of Y- chromosoom, onderzochten we de mogelijke impact van een extra X- of Y-chromosoom op vroege sociaal cognitieve functies en de leeftijdsgerelateerde dynamiek tijdens de vroege ontwikkeling. We onderzochten de volgende sociaal cognitieve functies: sociale oriëntatie, joint attention, emotieherkenning en Theory of Mind.

Sociaal oriënteren (visueel sociale aandacht) bij kinderen met een extra X- of Y-chromosoom

Allereerst richtten we ons op de mogelijke impact van het extra X- of Y-chromosoom op een basale sociaal cognitieve vaardigheid die zich typisch ontwikkelt tijdens de eerste levensjaren, namelijk visuele oriëntatie op sociale informatie. We onderzochten dit met behulp van eyetracking metingen gedurende de presentatie van sociale stimuli. De eyetracking resultaten lieten een impact van het extra X- of Y-chromosoom zien op visuele oriëntatie voor sociaal relevante informatie zoals gezichten en met name de ogen (Hoofdstuk 5). Deze verminderde oriëntatie op sociale informatie was het meest uitgesproken bij kinderen van drie jaar en ouder, en wanneer de sociale belasting door visuele stimuli hoog was (dat wil zeggen stimuli met meerdere gezichten). Deze verschillen tussen kinderen met een extra X- of Y-chromosoom en hun leeftijdsgenoten met betrekking tot oriëntatie voor sociale cues, werden ook gevonden in eyetracking metingen bij een sociale plot waarin twee personen wederzijds sociale informatie uitwisselden. Bij kinderen met een extra X- of Y-chromosoom in de leeftijd van één en twee jaar werden echter geen andere patronen in sociale aandacht/oriëntatie gezien (Hoofdstuk 4). De resultaten van deze eyetracking studies suggereren dat kinderen met een extra X- of Y-chromosoom vanaf de leeftijd van 3 jaar sociale relevante informatie verminderd waarnemen dan hun leeftijdsgenoten. Deze kwetsbaarheden in de visuele oriëntatie voor sociaal

relevante informatie die al vroeg in het leven aanwezig zijn, kunnen een kind de sociale input ontnemen die nodig is om sociale informatie optimaal te verwerken, wat de hersenontwikkeling en sociaal-cognitieve ontwikkeling negatief zou kunnen beïnvloeden (Mundy & Neal, 2000).

Joint attention bij kinderen met een extra X- of Y-chromosoom

Met behulp van eyetracking metingen vonden we kwetsbaarheden in het kunnen delen van sociale aandacht, oftewel 'joint attention' (gezamenlijke aandacht; Hoofdstuk 4). Joint attention is het vermogen om de aandacht tussen interactieve sociale partners met betrekking tot objecten of gebeurtenissen te coördineren met als doel het bewustzijn van deze objecten of gebeurtenissen te delen (Nation & Penny, 2008). Joint attention wordt in de literatuur in verband gebracht met de vroege rijping van het besef van kinderen dat andere personen intenties hebben, en is cruciaal voor het ontwikkelen van perspectief nemende vaardigheden, maar ook voor andere cognitieve vaardigheden zoals het verwerven en ontwikkelen van taal. We vonden verschillen in de nauwkeurigheid om spontaan de blik of een wijzend gebaar van een andere persoon te volgen tussen de kinderen met een extra X- of Y-chromosom en hun leeftijdsgenoten, wat wijst op problemen met joint attention bij kinderen met een extra X- of Y-chromosoom. Deze kwetsbaarheden met joint attention werden over de hele leeftijdsrange van 1 tot 7 jaar gezien (Hoofdstuk 4).

Emotieherkenning van gezichten en Theory of Mind bij kinderen met een extra X- of Y- chromosoom

Vanuit de literatuur is bekend dat in de typische sociaal cognitieve ontwikkeling de basale niveaus van sociale cognitie, zoals sociale aandacht en joint attention gedurende een reeks van ontwikkelingstappen rijpen naar meer complexe sociaal cognitieve vaardigheden, zoals de herkenning van emoties uit gezichtsuitdrukkingen en Theory of Mind (Soto-Icaza, Aboitiz, & Billeke, 2015). De herkenning van emoties geeft de mogelijkheid om de emotionele staat van anderen te detecteren, en is daarom belangrijk tijdens sociale interacties (Grossmann & Johnson, 2007). In de typische ontwikkeling rijpt vanaf de leeftijd vanaf 3 jaar het begrip van de complexiteit van sociale interacties die leidt tot 'false belief' (= het vermogen dat iemand een verkeerde opvatting kan hebben over de realiteit afhankelijk van het perspectief op de situatie;

(Wellman, Cross, & Watson, 2001; Devine & Hughes, 2014). Dit toenemende begrip van 'false belief' wordt weerspiegeld in de verschillende niveaus van Theory of Mind, zoals het niveau dat verwijst naar het begrip van tweede-orde overtuigingen en het niveau van herkenning van de invloed van eerdere ervaringen op mentale toestanden (Wellman, 2014). Bij jonge kinderen met een extra X- of Y-chromosoom vonden we kwetsbaarheden in emotieherkenning en Theory of Mind (Hoofdstuk 4 en 5).

Er werd een verschil gevonden in het vermogen tot emotieherkenning tussen kinderen met en zonder een extra X- of Y-chromosoom, duidend op tekortkomingen bij jonge kinderen met een extra X- of Y-chromosoom om emoties te herkennen uit gezichtsuitdrukkingen van anderen (Hoofdstuk 5). De percentages van jonge kinderen met een extra X- of Y-chromosoom die scoorden in de milde (8,5%) en ernstige klinische range (32,8%) zijn vergelijkbaar met percentages uit eerder onderzoek bij oudere kinderen en jongeren met een extra X- of Y-chromosoom (Van Rijn, de Sonneville, & Swaab, 2018; Samango-Sprouse et al., 2018; Van Rijn, Stockmann, Van Buggenhout, van Ravenswaaij-Arts, & Swaab, 2014b).

Vergelijkbare problemen werden gevonden in Theory of Mind, wat aangeeft dat jonge kinderen met een extra X- of Y-chromosoom moeite hebben met het begrijpen van de mentale staat van anderen. Het percentage van jonge kinderen met een extra X- of Y-chromosoom dat scoorde in de milde (29,5%) en ernstige klinische range (6,6%) laat zien dat er al vroeg in de ontwikkeling aanzienlijke verstoringen in Theory of Mind kunnen worden gevonden (Hoofdstuk 4). Voor zowel emotieherkenning als Theory of Mind vonden we dat, naarmate de leeftijd van kinderen toeneemt, het verschil tussen kinderen met een extra X- of Y-chromosoom en hun normaal ontwikkelende leeftijdsgenoten groter wordt, met toenemend meer verstoring bij oudere kinderen met een extra X- of Y-chromosoom. Deze bevindingen suggereren dat sociaal cognitieve kwetsbaarheden later in het leven van individuen met een extra X- of Y-chromosoom vooraf worden gegaan door een kwetsbare vroege sociaal cognitieve ontwikkeling.

'Growing into deficit'-fenomeen in vroeg sociaal cognitief functioneren bij kinderen met een extra X- of Y-chromosoom

Het fenomeen dat de neurocognitieve ontwikkeling steeds meer afwijkt van typisch

ontwikkelende leeftijdsgenoten naar mate de leeftijd toeneemt, zoals gevonden in dit proefschrift voor sociale oriëntatie, emotieherkenning en Theory of Mind (maar niet voor joint attention), wordt vaak gezien tijdens de ontwikkeling van kinderen met genetische syndromen en neurobiologische ontwikkelingsstoornissen. Dit fenomeen wordt 'growing into deficit' genoemd (zie bijvoorbeeld (Rourke, 1983; Sprong, 2008). Effecten van genetische variaties worden vaak later in de ontwikkeling zichtbaar, wanneer een kind wordt geconfronteerd met complexere ontwikkelingstaken en wanneer een kwetsbare rijping van het brein leidt tot een toenemende discrepantie met de leeftijdsnormen. Vanuit neuropsychologisch perspectief ontvouwt de functionaliteit van breinnetwerken zich als gevolg van neuro-anatomische rijping, wat tot uiting komt in toenemende neurocognitieve vermogens en gedragsmogelijkheden bij het zich ontwikkelende kind. De ontwikkeling van neurocognitieve functies vindt plaats in een relatief stapsgewijs patroon, waarbij de volgende stap afhankelijk is van voorgaande stappen. Vroege verstoringen van de neuro-anatomische groei en de vorming van interactieve hersennetwerken zoals het 'sociale brein', voor een belangrijk deel aangestuurd door genetische factoren zoals bijvoorbeeld het extra X- of Y-chromosoom (Raznahan et al., 2016), kunnen daarom van invloed zijn op de ontwikkeling van een opvolgende ontwikkelingsstap (Grossmann & Johnson, 2007). Echter, de impact van deze stoornissen kan pas later in de ontwikkeling tot cognitieve problemen leiden wanneer een ontwikkelingstaak wordt aangeboden waarvoor het brein niet volledig is uitgerust.

Functionele breinontwikkeling omvat de reorganisatie van interacties tussen verschillende corticale structuren en regio's, gebaseerd op een proces van specialisatie van de hersenen in steeds nauwkeuriger afgestemde functies (Johnson, 2001; Johnson et al., 2005). Complexere aspecten van sociale cognitie, zoals Theory of Mind, zijn bijvoorbeeld afhankelijk van de rijping van verschillende geassocieerde corticale gebieden. Dit corticale rijpingseffect van interactieve specialisatie, gedeeltelijk aangestuurd door genetische make-up, maakt het mogelijk dat de impact van verstoringen in de vroege rijping van het brein pas vele jaren later in de ontwikkeling zichtbaar wordt.

Op neurocognitief en gedragsniveau kunnen verstoringen in fundamentele sociaal cognitieve vaardigheden op jonge leeftijd, zoals gevonden in de studies die in dit proefschrift zijn opgenomen bij jonge kinderen met een extra X- of Y-chromosoom

(Hoofdstuk 4 en 5) leiden tot een cascade van negatieve ontwikkelingseffecten. Deze negatieve spiraal van toenemende kwetsbaarheden kan mogelijk van invloed zijn op de ontwikkeling van sociaal adaptief gedrag gedurende de kindertijd, de adolescentie en in de volwassenheid. Deze bevindingen impliceren dat een al vroeg gevonden kwetsbaarheid een voorbode kan zijn van een toenemende impact gedurende de levensloop.

Een longitudinaal perspectief: vroege sociale cognitie als voorspeller van latere ASS-symptomen

Kennis over vroege ontwikkelingspaden bij jonge kinderen met een extra X- of Y-chromosoom die leiden tot sociale kwetsbaarheden op latere leeftijd, is uiterst beperkt. Vanwege de bewezen ontwikkelingsrelatie tussen vroege sociale cognitieve vaardigheden (in het bijzonder joint attention) en de sociale (cognitieve) en communicatieve ontwikkeling (Franchini, Armstrong, Schaer, & Smith, 2019), onderzochten we of joint attention bij jonge kinderen met een extra X- of Y-chromosoom longitudinaal ernstige sociale beperkingen voorspelt, zoals weerspiegeld in ASS-symptomatologie.

De resultaten van deze studie (Hoofdstuk 6) lieten zien dat bij jonge kinderen met een extra X-of Y-chromosoom de neiging om de aandacht tussen sociale partners te coördineren (joint attention) voorspellend is voor ASS-symptomen op één jaar follow-up.

We vonden verschillen in de twee leeftijdsgroepen met betrekking tot het specifieke type joint attention bij het voorspellen van ASS-symptomen: terwijl alleen reageren op joint attention ASS-symptomen voorspelde bij 2 tot 4-jarige kinderen met een extra X- of Y-chromosoom, voorspelden *reageren* op en *initiëren* van joint attention ASS-symptomen bij kinderen van 4 jaar en ouder. De resultaten van deze studie geven aan dat problemen met joint attention mogelijk relevante markers zijn voor het ontstaan van ernstige sociale stoornissen en gerelateerd risico op ASS.

Effectiviteit van een neurocognitief trainingsprogramma op vroege sociale cognitie

Vanwege recente ontwikkelingen in de niet-invasieve prenatale testtechnologie (namelijk de introductie van de Non Invasieve Prenatale Test - NIPT; Samango-Sprouse, Keen, Sadeghin, & Gropman, 2017; Tartaglia et al., 2020), is het mogelijk om X- of Y-gebonden trisomieën al prenataal te identificeren, afhankelijk van de ethische en politieke normen in het specifieke land. Dat resulteert in een wereldwijd toenemend aantal diagnoses van trisomieën van het X- en Y-chromosoom. Deze snelle toename van prenatale diagnoses biedt de mogelijkheid om de impact van een extra X- of Y-chromosoom op de vroege ontwikkeling prospectief te onderzoeken, maar ook de dringende noodzaak om te onderzoeken of vroege preventieve interventies mogelijk het risico op problemen in sociaal cognitief functioneren kunnen verminderen (Herlihy & McLachlan, 2015).

Tot op heden is er zeer beperkt onderzoek gedaan naar de mogelijke effecten van niet-medische behandeling op de ontwikkeling van individuen met een extra X- of Y-chromosoom. Daarom wilden we in een pilot studie de werkzaamheid onderzoeken van een computer-gebaseerd neurocognitief trainingsprogramma bij 4-8 jaar oude kinderen met een extra X- of Y-chromosoom, gericht op het verbeteren van het begrip van sociale signalen in gezichtsuitdrukkingen. Er werden veelbelovende resultaten gevonden met betrekking tot de effectiviteit van de training, waaruit bleek dat het volgen van de 4-weekse neurocognitieve training bij kinderen met een extra X- of Y-chromosoom effectief was in het verbeteren van het vermogen om gezichtsuitdrukkingen te identificeren en te matchen (Hoofdstuk 7). Deze bevindingen waren onafhankelijk van het niveau van globaal cognitief functioneren en van het niveau van expressieve en receptieve taalvaardigheden. Na het voltooien van het trainingsprogramma vertoonden kinderen met een extra X- of Y-chromosoom emotieherkenningsvaardigheden op een niveau dat niet te onderscheiden was van leeftijdsgenoten zonder een extra X- of Y-chromosoom, daar waar de groep bij aanvang significant meer problemen had met emotieherkenning. Deze bevindingen illustreren dat er mogelijkheden zijn om de ontwikkeling van emotieherkenning bij kinderen met een extra X- of Y-chromosoom al op jonge leeftijd positief te ondersteunen. Gezien het bewijs dat bij kinderen met een extra X- of Y-chromosoom vroege sociaal-cognitieve kwetsbaarheden kunnen ontstaan en sterker aanwezig kunnen zijn als de leeftijd toeneemt, kan vroege ondersteuning van

de vroege sociaal-cognitieve ontwikkeling nadelige ontwikkelingstrajecten van jonge kinderen met een extra X- of Y-chromosoom mogelijk positief beïnvloeden (Mundy & Neal, 2001; Dawson, 2008).

Beperkingen van het huidige onderzoek en aanbevelingen voor vervolgonderzoek

Bij het interpreteren van de uitkomsten van het huidige proefschrift moet rekening worden gehouden met een aantal belangrijke kanttekeningen. De eerste beperking is het cross-sectionele design van de meeste studies die in dit proefschrift worden gepresenteerd, waardoor oorzaak-gevolg conclusies beperkt getrokken kunnen worden. Hoewel we wel de longitudinale en voorspellende waarde van joint attention voor latere ASS-symptomen hebben onderzocht, is de interpretatie van leeftijdsdynamische effecten gebaseerd op vergelijkingen van leeftijdscohorten in plaats van op een longitudinale beoordeling. Toekomstige studies moeten zich verder richten op de longitudinale ontwikkeling van sociaal adaptief functioneren bij kinderen met een extra X of Y chromosoom, die met prospectieve follow-up verder zal worden onderzocht in de populatie van kinderen met een extra X of Y chromosoom in de TRIXY Studie. Met behulp van longitudinale studies is het namelijk mogelijk om te onderzoeken of markers in vroege ontwikkeling, zoals bijvoorbeeld sociale oriëntatie maar ook andere factoren in domeinen van executief functioneren en/of taal, voorspellend zijn voor latere kwetsbare uitkomsten en klinische diagnoses van neurologische ontwikkelingsstoornissen, zoals ASS en aandachtstekort/hyperactiviteitsstoornis (ADHD).

Ten tweede wordt de meerderheid van de individuen met een extra X of Y chromosoom nog steeds niet gediagnosticeerd tijdens het leven (Berglund, Stochholm, & Gravholt, 2019). De groep gediagnosticeerde jonge kinderen groeit echter wereldwijd snel met de introductie van de NIPT die het mogelijk maakt om het extra X- of Y-chromosoom al voor de geboorte te diagnosticeren, afhankelijk van de ethische en politieke normen in het specifieke land. In het huidige proefschrift waren de uitkomsten met betrekking tot sociale cognitie en sociaal gedrag over het algemeen niet afhankelijk van de wervingsstrategie (prospectieve follow-up groep; informatie zoekende ouders groep; klinisch verwezen groep). Dat suggereert dat onze bevindingen representatief zijn voor deze groep gediagnosticeerde kinderen. Het blijft echter onzeker in welke mate de bevindingen in deze studie ook gegeneraliseerd kunnen worden naar

degenen die een extra X- of Y-chromosoom hebben, maar niet gediagnosticeerd zijn. Dit kan gaan om kinderen die géén zorgvraag hebben of om kinderen die wél een zorgvraag hebben, maar bij wie niet bekend is dat het extra X- of Y-chromosoom het onderliggende genotype is.

Ten derde hebben we jongens met 47,XXY (Klinefelter syndroom) in onze studie opgenomen, ongeacht of deze kinderen een behandeling met testosteron hadden gekregen. Voor zover wij weten, heeft één gerandomiseerde gecontroleerde studie (Randomized Control Trial; RCT) de resultaten van testosteronbehandeling beoordeeld op door ouders gerapporteerde sociale gedragsresultaten bij kinderen in de leeftijd van 4-12 jaar (Ross et al., 2017). In deze RCT werden verbeteringen gevonden na 24 maanden testosteronbehandeling bij door ouders gerapporteerde sociale problemen van hun kind. Hoewel een percentage jongens met 47,XXY in de huidige studie een testosteronbehandeling kreeg (46%), waren het design en de steekproef niet geschikt om de werkzaamheid van een testosteronbehandeling op de sociale ontwikkeling te bestuderen. Aangezien er sociaal (cognitieve) kwetsbaarheden werden gevonden in de gehele groep van kinderen met een extra X- of Y-chromosoom en dus ook 47,XXX en 47,XYY waarbij hypogonadisme/testosteron afwijkingen niet typerend zijn, suggereren de resultaten van dit proefschrift voorzichtig dat deze problemen met de sociaal adaptieve ontwikkeling niet volledig zijn toe te schrijven aan een testosteron tekort. Gerandomiseerde en placebo-gecontroleerde onderzoeken zouden de effecten van testosteronbehandeling op sociale (cognitieve) uitkomsten bij jonge kinderen met 47,XXY verder kunnen onderzoeken (zoals op dit moment wordt uitgevoerd in de volgende studie: PI Davis, NCT03325647).

Ten vierde zijn er nog onderzoeksvragen blijven liggen rondom achtergrond kenmerken, familiegeschiedenis en vroege omgevingservaringen die de sociaal adaptieve (cognitieve) ontwikkeling bij kinderen met een extra X- of Y-chromosoom kunnen beïnvloeden. De variabiliteit in fenotypes van individuen met een extra X- of Y-chromosoom biedt een unieke kans om niet alleen genetische maar ook omgevingsaspecten te onderzoeken die bijdragen aan neurocognitieve en gedrags-ontwikkelingsuitkomsten. Vroege omgevingsfactoren kunnen mogelijk leiden tot extra kwetsbaarheden in de neurocognitieve en gedragsmatige ontwikkeling. Het onderzoeken van deze gen-omgevingsinteracties vraagt om een integratief perspectief, rekening houdend met kwetsbaarheden in de ontwikkeling van kinderen

met een extra X- of Y-chromosoom in de context van omgevingservaringen. Voor zover wij weten, onderzocht één studie de invloed van vroege stressvolle levenservaringen in relatie tot sociaal-cognitieve uitkomsten bij kinderen met een extra X- chromosoom (Van Rijn, Barneveld, Descheemaeker, Giltay, & Swaab, 2018). De resultaten van deze studie laten zien dat 9-18-jarige kinderen met een extra X-chromosoom kwetsbaar zijn voor vroege 'life events', waarbij vooral de ontwikkeling van sociale cognitie werd beïnvloed, meer dan bij leeftijdsgenoten. Deze bevindingen ondersteunen de hypothese dat stress in het vroege leven de breinontwikkeling kan beïnvloeden bovenop de genetische kwetsbaarheid die aanwezig is door het extra X- of Y-chromosoom (Raznahan et al., 2016). Meer inzicht in vroege omgevingsaspecten, die de sociaal-cognitieve ontwikkelingsresultaten bij jonge kinderen met een extra X- of Y-chromosoom beïnvloeden, kan helpen bij het identificeren van beschermende factoren in de omgeving van kinderen. Deze omgevingsfactoren kunnen vervolgens worden ingezet bij het minimaliseren van de ontwikkelingsimpact als gevolg van het extra X- of Y-chromosoom.

Ten slotte hebben we ons in dit proefschrift vooral gericht op de impact van het extra X- of Y- chromosoom op sociaal adaptief functioneren. De resultaten van recente reviews van onze onderzoeksgroep (Van Rijn, 2019; Urbanus, Van Rijn, & Swaab, 2020) suggereren dat naast sociale cognitie, de domeinen taal, executieve functies en emotieregulatie ook kwetsbare domeinen zijn die een onderliggend risicomechanisme kunnen vormen bij gedragsproblemen. De kennis over de vroege impact van een extra X- of Y-chromosoom op domeinen van taal en executieve functies is momenteel groeiende (zie bijvoorbeeld Kuiper, Swaab, Tartaglia, & Van Rijn, 2021; Urbanus, Swaab, Tartaglia, Boada, & Van Rijn, 2022; Capelli et al., 2022). Omdat symptomen van psychopathologie worden veroorzaakt door een complex samenspel van neurocognitieve sterktes en zwaktes kan het interessant zijn om subgroepen van kinderen met een extra X- of Y-chromosoom te identificeren die een specifiek neurocognitief profiel delen op domeinen van taal, sociale cognitie, executief functioneren en emotieregulatie, onderliggende sociale stoornissen en gerelateerde psychopathologie. Inzichten in de interactie tussen neurocognitieve mechanismen onderliggend aan gedragsproblemen en gerelateerde psychopathologie kunnen meer gespecialiseerde informatie opleveren over ontwikkelingspaden die worden gedeeld door een subgroep van kinderen met een extra X- of Y-chromosoom (Borsboom & Cramer, 2013).

Ondanks deze beperkingen kunnen we met vertrouwen concluderen dat het gedragsmatig sociaal adaptief functioneren en onderliggende sociaal-cognitieve mechanismen kwetsbaar zijn in de vroege ontwikkeling van jonge kinderen met een extra X- of Y-chromosoom. Dit leidt tot implicaties voor de klinische praktijk.

Implicties voor de klinische praktijk

De bevindingen zoals beschreven in dit proefschrift onderstrepen het belang van het nauw monitoren van sociaal adaptief gedrag en cognitief functioneren bij kinderen met een extra X of Y chromosoom als routine in de klinische zorg in de eerste levensjaren. Omdat we ontdekten dat joint attention voorspellend is voor ernstige sociale beperkingen, zoals weerspiegeld in ASS-symptomen, kunnen problemen met joint attention dienen als een vroege marker voor een risicovol ontwikkelingsprofiel. Het is om verschillende redenen relevant om vroeg kinderen op te sporen en te ondersteunen die risico lopen op een verstoorde sociale ontwikkeling.

Als eerste ervaren ouders van kinderen met een diagnose van een extra X of Y chromosoom vaak stress en onzekerheid over de ontwikkeling van hun kind en hun eigen opvoedingsvaardigheden (Richardson, Riggan, & Allyse, 2021). Vroegtijdige opsporing van ontwikkelingsrisico's bij kinderen leidt tot passende ondersteuning van ouders door middel van het aanbieden van psycho-educatie en/of ouderbegeleiding. Vroege ondersteuning van ouders zou kunnen leiden tot vermindering van stress en onzekerheid en een verbeterd functioneren van ouders door het aanleren van effectieve copingstrategieën (zie voor voorbeelden over de effectiviteit van vroege ouderbegeleiding in de ASS-literatuur: Estes, Swain, & MacDuffie, 2019; Saccà, Cavallini, & Cavallini, 2019), dat naast het positieve effect daarvan op het psychisch welzijn van ouders ook gunstig is voor de ontwikkeling van het kind.

Ten tweede hebben kinderen met sociale beperkingen minder sociale leerervaringen dan hun leeftijdsgenoten. Dit kan leiden tot een cascade van negatieve ontwikkelingseffecten. Ter illustratie, verminderde sociale aandacht kan leiden tot beperkte kwantitatieve en kwalitatieve mogelijkheden om sociale kennis te verwerven bij kinderen met een extra X- of Y-chromosoom en om te leren van (complexe) sociale interacties (Mundy & Neal, 2001). Door aandacht te besteden aan

het gezicht en de ogen van een ander en sociale input te begrijpen en interpreteren kunnen typisch ontwikkelende kinderen rijke sociale ervaringen opdoen die cruciaal zijn voor de ontwikkeling van sociale en communicatieve vaardigheden, zoals joint attention, taalverwerving en gezichts- of emotieherkenning (Gliga & Csibra, 2007). Een verminderde aandacht voor de ogen en gezichten van anderen kan daarom een brede impact hebben op de complexe ontwikkeling van sociale (cognitieve) vaardigheden, die gebaseerd zijn op basale sociaal-perceptuele informatie. Het is daarom van belang om de ontwikkeling van sociale communicatie al vanaf jonge leeftijd nauwlettend te monitoren. Deze monitoring van kwetsbare kinderen met een extra X- of Y- chromosoom en daarop gebaseerde vroege ondersteuning en interventie op maat kunnen de sociaal adaptieve ontwikkeling tijdens de kindertijd positief beïnvloeden.

Negatieve cascade-effecten in de ontwikkeling zouden kunnen verklaren waarom en hoe kwetsbaarheden in de vroege kinderjaren kwetsbaarheden of zelfs beperkingen op verschillende ontwikkelingsdomeinen in de kindertijd, adolescentie en volwassenheid voorspellen. Gezien de effecten die zich in de loop van de tijd op het sociale adaptieve domein kunnen verspreiden, kunnen gerichte interventies die tijdens de ontwikkeling op belangrijke momenten worden ingezet, negatieve cascades onderbreken of positieve cascades bevorderen (Masten & Cicchetti, 2010). Preventieve ondersteuning van sociale vaardigheden en vroege behandeling van kinderen met een extra X- of Y-chromosoom is daarom essentieel. In dit proefschrift werd een veelbelovend effect gevonden van een neurocognitieve training gericht op herkenning van emoties op gezichten (zie Hoofdstuk 7). Een 4-weekse training in de thuissituatie bleek effectief te zijn in het verbeteren van het vermogen om gezichtsuitdrukkingen te identificeren en te matchen bij 4-8-jarige kinderen met een extra X- of Y- chromosoom. Deze bevinding illustreert dat er al op jonge leeftijd mogelijkheden zijn om de sociaal-cognitieve ontwikkeling van kinderen met een extra X- of Y-chromosoom positief te ondersteunen. Omdat neurocognitieve trainingsprogramma's gemakkelijk toegankelijk en kosteneffectief zijn, kan ondersteuning van de vroege neurocognitieve ontwikkeling preventief worden gebruikt in thuis- en schoolomgevingen.

Neurocognitieve trainingsprogramma's kunnen ook worden ingezet als onderdeel van een op maat gemaakt en integratief interventieprogramma voor jonge kinderen met

een extra X- of Y-chromosoom op basis van een individueel vastgesteld neurocognitief sterkte-zwakte profiel. Vroege ondersteuning van de sociaal cognitieve ontwikkeling kan ontwikkelingstrajecten van jonge kinderen met een extra X- of Y-chromosoom ondersteunen waardoor de negatieve langetermijneffecten van een extra X- of Y-chromosoom op sociaal adaptief functioneren mogelijk verzacht kunnen worden (Mundy & Neal, 2001; Dawson, 2008).

Ten derde, omdat vroege gedragstherapeutische interventies veel gebruikt worden om de vroege ontwikkeling van kinderen met ASS en hun families te ondersteunen (zie voor een overzicht: French & Kennedy, 2018), kunnen deze vroege interventies mogelijk ook gunstig zijn voor jonge kinderen met een extra X- of Y-chromosoom (Tartaglia et al., 2017). Een aantal behandelprogramma's is gunstig gebleken voor jonge kinderen met ASS of een risico op ASS, zoals bijvoorbeeld Applied Behavioural Analysis (ABA), Early Start Denver Model, Floortime, Pivotal Response Treatment (PRT) en Verbal Behavior Therapy (Bishop-Fitzpatrick, Minshew, & Eack, 2014; Cadogan & McCrimmon, 2015; French & Kennedy, 2018; Pajareya, Sutchritpongsa, & Kongkasuwan, 2019). Het is belangrijk om de mogelijke werkzaamheid en langetermijnresultaten van deze interventies en programma's verder te onderzoeken in populaties van kinderen met een extra X- of Y-chromosoom, vooral gezien de noodzaak om ondersteunende preventieve interventies te kunnen aanbieden aan de groeiende groep (prenataal) gediagnosticeerde kinderen met een extra X- of Y-chromosoom (Samango-Sprouse, Keen, Sadeghin, & Gropman, 2017; Tartaglia et al., 2020).

Ten slotte, er is behoefte aan gespecialiseerde interdisciplinaire zorg om de ontwikkeling van kinderen met een extra X- of Y-chromosoom te monitoren en te ondersteunen. Hoewel er variabiliteit is in het fenotype van individuen met een extra X- of Y-chromosoom, is het extra X- of Y-chromosoom niet alleen geassocieerd met kwetsbaarheden in sociaal adaptief functioneren, maar ook met kwetsbaarheden die gerelateerd zijn aan sociaal adaptief functioneren zoals emotieregulatie, cognitief functioneren, executief functioneren, receptieve en expressie taalontwikkeling en adaptieve vaardigheden. Bovendien kunnen medische aandoeningen zoals hypogonadisme bij het 47,XXY/Klinefelter-syndroom de sociale ontwikkeling van kinderen met een extra X-chromosoom mogelijk beïnvloeden (Tartaglia et al., 2015; Van Rijn, 2019). Momenteel worden kinderen en jongeren met een extra X- of Y-

chromosoom vaak ondersteund door klinische genetici in ziekenhuizen waar families informatie krijgen over de genetische aandoening na (prenatale) diagnose en door kinderendocrinologen die ervaring hebben met testosteronbehandeling in 47,XXY (Gravholt, Tartaglia, & Disteche, 2020). Naast genetische en endocrinologische zorg is het echter van belang om ook tijdig neurocognitieve ontwikkelingsproblemen te monitoren en de neurocognitieve ontwikkeling te ondersteunen. Gecoördineerde multidisciplinaire zorg is dus belangrijk voor het bieden van gespecialiseerde diagnostiek en behandeling van kinderen en jongeren met een extra X- of Y-chromosoom; deze zorg wordt bijvoorbeeld geboden in het Nederlandse TRIXY Expertisecentrum (www.trixyexpertisecentrum.nl; Leiden, Nederland). Evidence-based internationale consensus over zorgleidraden zijn cruciaal om zorg van de beste kwaliteit te bieden aan kinderen en jongeren met een extra X- of Y-chromosoom en hun families.

Conclusie

Concluderend, de resultaten gepresenteerd in het huidige proefschrift laten zien dat een extra X- of Y-chromosoom al zeer vroeg in de ontwikkeling samenhangt met kwetsbaarheden in sociaal adaptief functioneren en onderliggende vroege sociaal cognitieve vaardigheden (zie Box 1 voor de belangrijkste conclusies van dit proefschrift). De bevindingen laten zien dat een extra X- of Y-chromosoom de sociale gedragsontwikkeling vanaf de eerste levensjaren kan beïnvloeden, wat zich uit in kwetsbaarheden in de vroege sociale communicatie en sociaal teruggetrokken gedrag tijdens sociale interacties. Vroege sociaal cognitieve disfuncties die ten grondslag kunnen liggen aan de sociaal adaptieve kwetsbaarheden in kinderen met een extra X- of Y-chromosoom zijn onder meer sociale oriëntatie, joint attention en meer complexe sociaal cognitieve vaardigheden zoals het begrijpen van emotionele gezichtsexpressies en Theory of Mind. Deze resultaten laten zien dat het nuttig is om vroege ontwikkelingspaden van kinderen met een extra X- of Y-chromosoom prospectief te onderzoeken, aangezien het inzicht geeft in gedragsuitkomsten en onderliggende neurocognitieve mechanismen van populaties met een verhoogd risico op sociale uitdagingen en bijbehorende psychopathologie. Ook suggereren de resultaten een profiel van sociale (cognitieve) kwetsbaarheden bij jonge kinderen met een extra X- of Y-chromosoom, wat vroege monitoring en indien nodig gerichte interventie van de sociaal adaptieve ontwikkeling noodzakelijk maakt. Ook werd

duidelijk dat er mogelijkheden zijn om de ontwikkeling van sociale cognitie, namelijk de herkenning van gezichtsemoties, positief te ondersteunen bij jonge kinderen met een extra X- of Y-chromosoom.

Kwetsbaarheden in de sociaal adaptieve ontwikkeling hebben grote invloed op de kwaliteit van leven (de Vries & Geurts, 2015). Daarom pleiten de bevindingen beschreven in dit proefschrift voor vroege en nauwlettende monitoring van sociaal gedrag en cognitieve ontwikkeling en van risico's op sociale beperkingen gerelateerd aan ASS, gedurende de vroege kinderjaren. Vroege implementatie van (preventieve) ondersteuning van het kind en ouders en interventie ter ondersteuning van de sociale ontwikkeling heeft het potentieel om de impact van het extra X- of Y- chromosoom te verminderen, waardoor de kwaliteit van leven van kinderen met een extra X- of Y- chromosoom en hun families kan worden vergroot.

Box 1. Conclusies van het huidige proefschrift

Conclusies van het huidige proefschrift

De belangrijkste bevindingen van de zes studies die worden gepresenteerd in het huidige proefschrift kunnen als volgt worden geformuleerd:

1. Een extra X- of Y-chromosoom heeft invloed op de sociaal adaptieve gedragsontwikkeling vanaf de eerste levensjaren, weerspiegeld in kwetsbaarheden in vroege sociale communicatie en sociale interactie. Kwetsbaarheden in sociale interactie zijn onder meer lagere frequenties van oogcontact, meer problemen met het gebruiken en reageren op non-verbale uitingen van verzoeken, verminderd reageren tijdens sociale interacties en meer sociale terugtrekking, met name wanneer de sociale eisen vanuit de omgeving hoog zijn (Hoofdstuk 2 en 3).
2. Vroege sociaal cognitieve disfuncties die aan de basis kunnen liggen van een kwetsbare sociale ontwikkeling bij kinderen met een extra X- of Y-chromosoom, zijn visuele oriëntatie op sociale signalen, joint attention, emotieherkenning en Theory of Mind (Hoofdstuk 4 en 5).
3. De vroege sociaal cognitieve ontwikkeling van kinderen met een extra X- of Y-chromosoom wordt gekenmerkt door een fenomeen van 'growing into deficit' (Hoofdstuk 4 en 5), zoals zichbaar in de kwetsbare ontwikkeling van emotieherkenningsvaardigheden en Theory of Mind.
4. Kinderen met een extra X- of Y-chromosoom lopen een verhoogd risico op symptomatologie geassocieerd met ASS, waarbij 22% van de kinderen boven de klinische cutoff scoort op zowel het gebied van sociale interactie en communicatie als beperkte interesses en repetitief gedrag (Hoofdstuk 3 en 6).
5. Joint attention is gerelateerd aan symptomen van ASS bij een follow-up van één jaar, en dient mogelijk als een vroege marker voor kinderen met een extra X- of Y-chromosoom die risico lopen op ernstige sociale beperkingen (Hoofdstuk 6).
6. Neurocognitieve training van emotieherkenningsvaardigheden heeft het potentieel om de impact van het extra X- of Y-chromosoom op vroege sociaal cognitieve vaardigheden te verminderen (Hoofdstuk 7).

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CURRICULUM VITAE

Curriculum Vitae

Nienke Bouw werd geboren op 9 januari 1989 in Barneveld. Na het behalen van het Gymnasium diploma aan het Van Lodenstein College te Amersfoort in 2007, rondde zij in 2012 de opleiding Social Work (Maatschappelijk Werk en Dienstverlening) aan de Christelijke Hogeschool in Ede af. Ze werkte vervolgens als (ambulant) begeleider in de zorg voor mensen met een verstandelijke beperking en kinderen en jongeren met een Autisme Spectrum Stoornis bij Philadelphia Zorg en Ipse de Bruggen.

In 2014 startte ze met de tweejarige Research Master 'Developmental Psychopathology in Education and Child Studies' aan de Universiteit Leiden. Gedurende deze master was Nienke betrokken bij diverse onderzoeksprojecten gericht op het begrijpen van de invloed van risico factoren op de sociale ontwikkeling van jonge kinderen, onder begeleiding van dr. Stephan Huijbregts en dr. Szilvia Biro. Ook deed ze een klinische stage bij het Ambulatorium, polikliniek van de Universiteit Leiden, onder begeleiding van dr. Yvette Dijkxhoorn. Tijdens deze stage behaalde ze de Basisaantekening Diagnostiek.

In 2016 studeerde Nienke cum laude af en startte ze als junior onderzoeker aan de afdeling Neuropedagogiek en Ontwikkelingsstoornissen van de Universiteit Leiden. In 2017 begon ze haar promotie onderzoek, onder begeleiding van dr. Sophie van Rijn en prof.dr. Hanna Swaab. De centrale doelstelling van dit onderzoek was het bestuderen van de impact van een genetische variatie (extra X-of Y-chromosoom) op sociaal adaptief gedrag en de sociaal cognitieve ontwikkeling, met als doel het begrijpen en identificeren van vroege markers van ontwikkelingsrisico's die leiden tot sociale kwetsbaarheden en bijbehorende psychopathologie. De resultaten van dit onderzoek staan beschreven in het proefschrift dat voor u ligt en presenteerde Nienke op diverse (inter-)nationale congressen. Twee keer werd een posterprijs toegekend voor haar onderzoek: op het Nationaal Autisme Congres in 2021 en bij de Nederlandse Vereniging voor Neuropsychologie in 2022.
Naast haar aanstelling als promovendus werkte zij gedurende het gehele promotie

traject als docent binnen het bachelor- en masteronderwijs aan de afdeling Neuropedagogiek en Ontwikkelingsstoornissen. Sinds de afronding van haar proefschrift is Nienke werkzaam als orthopedagoog bij de Polikliniek Kinder- en Jeugdpsychiatrie van het Erasmus MC/Sophia Kinderziekenhuis in Rotterdam.

Beeld: Suédy Maricio

LIST OF PUBLICATIONS

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Dankwoord

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