Neurodevelopmental Impact of Sex Chromosome Trisomy in Young Children: The Regulation of Emotion, Cognition, and Behavior

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The research reported in this dissertation was funded by:

Nederlandse Organisatie voor Wetenschappelijk Onderzoek

Promotores

Dr. S. (Sophie) van Rijn

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CHAPTER 1

General introduction

General Introduction

Sex chromosome trisomies (SCT) are one of the most common chromosomal aneuploidies in humans (Hong & Reiss, 2014) with an estimated prevalence around 1 in 650 to 1,000 live births (Berglund et al., 2019; Bojesen et al., 2003; Groth et al., 2013; Morris et al., 2008). Individuals with SCT have an increased risk for psychopathology, which refers to social, emotional, cognitive, and behavioral problems (Giltay & Maiburg, 2010; Groth et al., 2013; Tartaglia et al., 2010). By viewing behavior on a continuum ranging from "adaptive" to "non-adaptive", having significant behavioral problems can tremendously impact adaptive day-to-day functioning to such an extent that the behavioral problems can also be classified as symptoms of psychiatric classifications (according to the DSM-5, APA, 2013). For individuals with SCT, an increased risk for psychiatric classifications has been described (van Rijn, 2019), including affective disorders, social-communicative disorders, and neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). However, not all individuals with SCT are equally impacted and variability in outcome is rather rule than exception. To understand this heterogeneity in outcomes and how the extra sex chromosome impacts development, it may be helpful to implement a neurocognitive perspective. Individual differences in neurocognition (e.g., information processing skills anchored in brain functioning) could help explain individual differences in outcomes and henceforth the increased risk for psychopathology (Anderson, 2001). Within this dissertation, the aim is to identify early neurocognitive risk factors in young children with SCT, with a specific focus on those skills crucial to social, emotional, and behavioral adaptation. The regulation of thoughts, emotions, and behavior in the context of others, in other words *self-regulation*, is a tremendous influential factor when it comes to daily life functioning and therefore an interesting candidate to study in relation to psychopathology.

The fact that SCT can be diagnosed as early as pregnancy has important additive value in increasing our understanding of underlying mechanisms related to psychopathology. Studying genetic populations from an early age provides an unique opportunity to examine at-risk

development prospectively before psychopathology actually enfolds. These “neurogenetic” studies include a bottom-up approach that may provide insight in those neurocognitive building blocks essential to childhood development to explain how individual differences in early development could lead to psychopathology later in life (Reiss & Dant, 2003). It complements traditional psychopathology research (top-down approach), where specific neurocognitive profiles are identified in individuals diagnosed with a neurodevelopmental disorder (classified according to the DSM-5), such as ASD and ADHD. Increasing knowledge of developmental pathways in children with SCT would thus not benefit only individuals with SCT and their families, but also other individuals that suffer from neurodevelopmental problems. The study of SCT would serve as a ‘high risk model’ to understand the etiology of psychopathology and to pinpoint targets for early preventive support (Beauchaine, 2009), which would also sharpen our knowledge on gene-brain-behavior pathways in neurodevelopmental disorders.

The TRIXY Early Childhood Study

This dissertation and its included studies are part of a larger study, The TRIXY Early Childhood Study, that investigates neurocognitive mechanisms (e.g., language, social cognition, and self-regulation) in young children with SCT in order to explain the increased risk for psychopathology. One of the key objectives is to link biomarkers and neurocognition to daily life functioning by using sensitive, state-of-the-art measures (e.g., early neurocognitive performance tests, eye tracking, physiology). Furthermore, it evaluates the effectiveness of intervention tools targeted at stimulating neurocognitive development and thereby potentially reducing the risk for later psychopathology. The TRIXY Early Childhood Study includes a large international cohort of children with SCT ($n > 100$) between the ages of 1 to 7 years old, for which data was collected on their development at different timepoints (including a 1 year follow-up) and compared to data of peers from the general population ($n > 100$). Children with SCT and their parents were recruited through the Centre of Expertise for Trisomy of the X and Y chromosomes (TRIXY) in the Netherlands and the eXtraordinarY Kids Clinic in Developmental Pediatrics at the Children's Hospital Colorado

(CHCO) in the United States of America (USA). How families came to learn of the study was recorded to correct for potential recruitment bias. This is important whilst studying genetic conditions, because studies need to include enough participants to cover the full range of potential outcomes and not only those who learned upon the diagnosis because of developmental difficulties, e.g. introducing potential bias in results (Prasad & James, 2009). The design of the TRIXY study, that included predominantly prenatally diagnosed children, aimed to tackle this and allowed for the unique and prospective investigation of an at-risk development before psychopathology enfolds.

General Background on Chromosomes and SCT

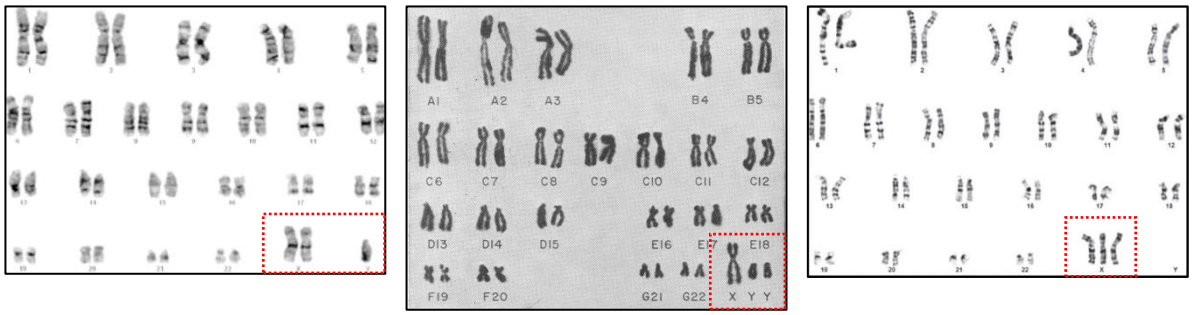
To understand the genetic background of children with SCT, a short description of typical genetics in humans is needed. Typically, humans are born with 22 pairs of chromosomes numbered from 1 to 22 along with 1 pair of sex chromosomes (X or Y chromosomes) adding up to a total of 46 chromosomes. A female carries two X chromosomes (46,XX) and a man usually carries one X and one Y chromosome (also noted as 46,XY).In males, the X chromosome always originates from the egg cell of the mother. The sex of the baby is determined by the sex chromosome transmitted in the sperm cell of the father. That cell can contain either an X or a Y chromosome. If the sperm contains the X chromosome, the embryo will carry a female karyotype (46,XX) and if the sperm contains the Y chromosome, the embryo will carry a male karyotype (46,XY). In the case of chromosomal aneuploidies (i.e., an atypical amount of chromosomes), a chromosomal pair includes more than two chromosomes. The presence of three chromosomes is called a trisomy. Whereas other chromosomal trisomies are usually associated with severe medical, cognitive, and functional impairments, SCT have typically more mild phenotypical outcomes. Remarkably, SCT are significantly less known in the general population and by clinicians, although it has a higher prevalence rate than other chromosomal trisomies, such as Down's syndrome (a trisomy of the 21st chromosomes). SCT has an estimated prevalence around 1 in 500 to 1,000 live births (Berglund et al., 2019), whereas Down's syndrome has a prevalence of 1 in 800 (Bittles et al., 2007). Despite its high prevalence, many individuals with SCT experience a significant delay in diagnosis or even non-diagnosis throughout

life. The estimated percentage of individuals that remain undiagnosed ranges from 75% to 88% (Berglund et al., 2019; Bojesen et al., 2003). Potentially related to this high non-diagnosis rate are the mild, variable, and mostly non-specific physical features, including minimal facial dysmorphisms, tall stature, and abnormal muscle tone (hypotonia)(Tartaglia et al., 2020).

The trisomy of the sex chromosomes is a *de novo mutation* and caused by random errors in cell division (Maiburg et al., 2012). Karyotypes that result from SCT are 47,XXY (Klinefelter's syndrome) and 47,XYY (XYY syndrome) in males, and 47,XXX (Trisomy X syndrome) in females. Trisomy karyotypes include 47 chromosomes (instead of the typical 46 chromosomes) and thus lead to the genetic notation of 47,XXY, 47,XXX and 47,XYY (see Figure 1 for the visual representation). Other more rare variants of additional chromosomes include karyotypes that have more than one additional chromosomes (for example 48,XXYY or 48,XXXY). In this dissertation, the focus is on sex chromosomes trisomies (SCT).

Figure 1

Example Karyotypes of Sex Chromosome Trisomies



A B C

Note. A: Karyotype of 47,XXY (Nagvenkar et al., 2005); B: Karyotype showing 47,XYY (Sandberg et al., 1963); C: Karyotype of 47,XXX (Kanaka-Gantenbein et al., 2004).

A Neurocognitive Perspective on SCT

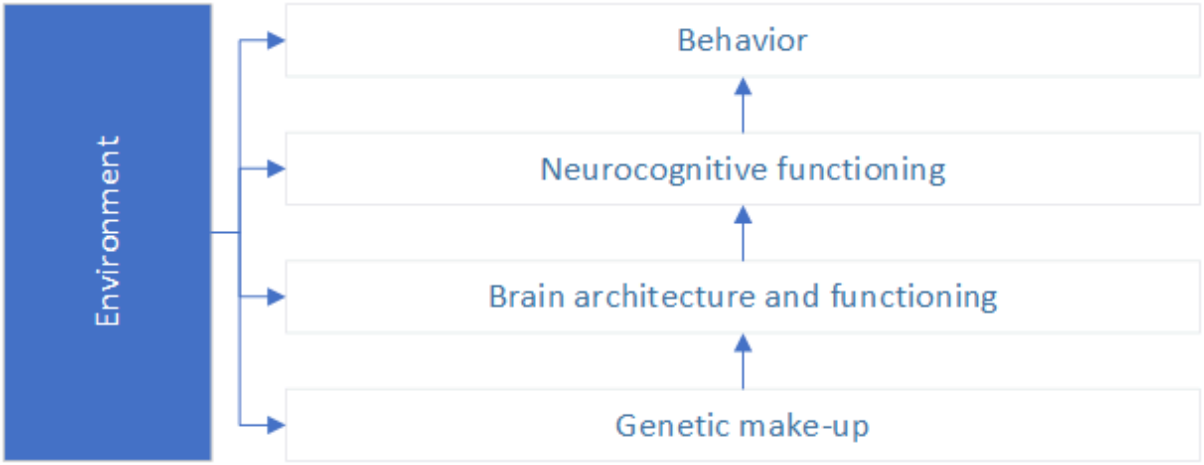
How a genetic condition such as sex chromosomal trisomy impacts daily life functioning is determined by many different factors and would include the somatic, cognitive, and psychosocial experiences that interact together to influence development (Anderson, Northam, & Wrennall, 2019). The research on SCT has traditionally focused on examining the somatic and physical features, with only a small proportion of the studies (25%) looking at social, emotional, and behavioral problems (Pieters et al., 2011). Even fewer studies examined neurocognitive outcomes in SCT, which is somewhat surprising, given that a significant fraction of genes on the sex chromosomes are associated with brain development and functioning (Zechner et al., 2001), suggesting a link between sex chromosomal aneuploidies and neurocognitive outcomes.

A neurocognitive approach could be relevant in explaining the link between the genetic make-up of children with SCT and the increased behavioral problems observed in daily life. A useful bottom-up approach model to describe how genetics might interact with environmental factors to shape behavioral outcomes is the brain-behavior model (Figure 2). An individual's genetic make-up is reflected in both the architecture of the brain as well as the functioning of the brain. As a result of both architecture and functioning of the brain, neurocognitive functions are developed and reflect the ability to process information. In turn, the complex interplay of multiple neurocognitive functions shape behavior, suggesting that neurocognitive factors can be viewed as building blocks for behavioral outcomes. Finally, environmental factors can influence all levels of the model, acting as either facilitative factors or barriers (Swaab et al., 2011).

Thus, as a result of (the complex interplay between) neurocognitive deficits, observable behavioral problems may arise. In addition, these problems can also be clustered based on a set of symptoms as a psychiatric classification according to diagnostic manuals, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM-5, American Psychiatric Association [APA], 2013). For example, the inability to maintain your attention (neurocognitive deficit) would be observed in daily life as the inability to finish homework and fail the accompanying test (behavioral problem). For

Figure 2

The Brain-Behavior Pathway Model (Swaab et al., 2011)



some children, these neurocognitive deficits and behavioral problems will significantly impact their day-to-day life to such an extent that a psychiatric classification also applies, for example severe symptoms of inattentiveness, hyperactivity, and impulsivity that are also part of the criteria for Attention-Deficit Hyperactivity Disorder (ADHD) according to the DSM-5 (APA, 2013).

Reflecting on the existing literature, multiple levels of the brain-behavior pathway model seem to be impacted in SCT. Studies already confirmed that brain differences and dysfunction are likely part of the SCT profile. For example, neuroimaging studies showed that brain architecture and functioning appears different in individuals with SCT compared to peers from the general population (Steinman et al., 2009, Warling et al., 2020). Especially relevant are those studies that found differences in areas of the brain known to be involved in processing social and emotion information, including the amygdala, the insula, the fusiform gyrus, and the superior temporal sulcus (van Rijn, Swaab, et al., 2012). In addition, neurocognitive differences are identified in the area of general intellectual functioning (albeit at the lower end of the typical range), social cognition, executive functioning, and language in school-aged children, adolescents, and adults with SCT (for a review on this topic, see van Rijn, 2019). Far less is known about the early development of neurocognitive skills in children with SCT (Urbanus, van Rijn, et al., 2020), but this type of research is a relatively new but

booming field. Part of the trend is the generic interest in examining neurocognitive profiles in relation to psychopathology and psychiatric conditions as well as newly developed techniques to measure neurocognition early in life (Kavanaugh et al., 2020). To conclude, a neurocognitive perspective on SCT (linking information processing deficits in the brain to behavioral outcomes) would be considered highly relevant when trying to understand developmental pathways into psychopathology.

Self-Regulation

One of the key neurocognitive functions to study in early childhood is that of self-regulation. **Self-regulation** refers to regulation of thoughts, emotions, attention, behavior, and impulses in order to meet goals and adequately respond to the environment (Blair & Diamond, 2008). Self-regulatory skills are of great importance with regards to daily functioning and quality of life, given that optimal self-regulation promotes positive adjustment and adaptation, as reflected in positive relationships, productivity, achievement, and a positive sense of self (Blair & Diamond, 2008). From a developmental perspective, studies have shown that self-regulation is associated with important long-term outcomes such as mental health (Moffitt et al., 2011), social competence (Bradley & Corwyn, 2013, 2007), and academic achievement (Eisenberg et al., 2010; Vazsonyi & Huang, 2010), showing that self-regulation is a vital skill to be acquired throughout childhood and adolescence. In fact, self-regulation is considered a transdiagnostic feature of psychopathology (Romer et al., 2021). In early childhood, impairments in the regulation of thoughts, emotions, and behavior are associated to adverse developmental outcomes, including internalizing and externalizing behavior problems (Kostyrka-Allchorne et al., 2020; Lemery-Chalfant et al., 2008).

The central aim of this dissertation was to explore neurocognitive aspects of self-regulation by combining new, sensitive, and direct measures in a large cohort of young children with SCT during a critical period of development, to determine how children with SCT perceive, process, experience, express, and cope with challenging situations. This dissertation focuses on three important

interrelated elements of self-regulation: behavioral regulation, cognitive regulation (in terms of executive functioning), and emotion regulation.

Regulation of Behavior

Being able to regulate your feelings and actions is an important part of social and adaptive functioning: It enables us to make good choices in accordance with our goals, but also to consider the feelings and actions of others around us and adjust our behavior accordingly. Self-regulation is precisely the reason why we are able to finish an important assignment for school or job, even when other activities are tempting. It is also the reason why we stop and think when a friend asks our opinion on their clothes. However, having difficulty regulating your actions can result in behavior that is maladaptive or socially inappropriate, for example unable to resist binge-watching your favorite Netflix show and failing to finish your assignment, or commenting on how ugly the clothes are without considering your friend's feelings. When these instances of poor self-regulation occur too frequently, it can significantly impact someone's ability to function in day-to-day social, academic, and occupational situations during lifetime. Thus, impaired self-regulation can result in behavior problems representing symptoms of many psychiatric/mental disorders (American Psychiatric Association, 2013). With this in mind, studying these types of symptoms have the potential to inform us on the development of self-regulation and its difficulties in early childhood.

The most salient markers of impaired self-regulation to study in early childhood are those behavioral symptoms associated with ADHD: a neurodevelopment disorder characterized by severe symptoms of inattentiveness, hyperactivity, and impulsivity that interfere with daily functioning and development (DSM-5: APA, 2013). In addition, ADHD-symptoms are an ideal candidate to study in children with SCT as a marker for self-regulation, given elevated clinical levels of ADHD-symptoms are present across all three karyotypes and across the broad age-range from school-age to adulthood (van Rijn, 2019). Information on early childhood (before the age of 6) is however not yet available. To be specific, within the TRIXY Early Childhood Study, we are not interested in classifying

these young children with SCT as having ADHD or not. In contrast, ADHD symptoms are viewed as a continuous measure to examine the type and variety in self-regulatory skills in early childhood. In other words: The presence and variety of ADHD symptoms would reflect individual differences (or deficits) in self-regulatory skills.

Regulation of Cognition (Executive Function)

Another important component of self-regulation is the cognitive ability to act purposefully and goal-directed, which is supported by our executive functions. The term *executive functions* (EF) refers to a set of interrelated neurocognitive skills essential to learn, cope, and manage daily life (Diamond, 2013). Several components can be identified, including attention, inhibition, monitoring, flexibility, working memory, planning, and fluency (Anderson, 2001). Proper executive functions are crucial when it comes to positive childhood development: executive functions promote mental and physical health; predict success in school and in life; and support cognitive, social, and psychological development (Diamond, 2013). On the other hand, impairments across executive functions are involved in many neurodevelopmental disorders, including ADHD (Diamond, 2005), ASD (Demetriou et al., 2018), and intellectual disabilities (Lee et al., 2015).

Until now, studies that have examined executive functions in individuals with an extra X or Y chromosome showed reduced executive function performance compared to population-based controls (for review see Urbanus et al., 2020 and Van Rijn, 2019). Children with SCT show more impairments across executive functions, including attention, inhibition, mental flexibility, working memory, and planning/problem solving (Janusz et al., 2020; Lee et al., 2015; Ross et al., 2008, 2009; Samango-Sprouse et al., 2018; van Rijn & Swaab, 2015). Furthermore, these impairments have been linked to increased externalizing behavior problems (van Rijn & Swaab, 2015), increased social difficulties (Skakkebæk et al., 2017) as well as increased symptoms of ASD (van Rijn et al., 2012), psychotic symptoms such as disorganized thought (Van Rijn et al., 2009), and ADHD symptoms (Lee et al., 2011). These studies show that early differences in neurocognition can have predictive value to later psychopathology in SCT, highlighting the importance of early investigation of skills yet in

development. To date, no other studies have examined early emerging executive functions in young children with SCT, especially none that have used age-sensitive neurocognitive assessments in addition to behavioral report data. Our study will be the first to do so.

Regulation of Emotions

The final component important to self-regulation is the ability to regulate our emotions. **Emotion regulation** refers to all processes that influence the occurrence, intensity, duration, and expression of emotions (Gross, 2013). Emotions provide us with key information on how to perceive the world around us (information-oriented), how to accomplish our goals (goal-oriented), and how to respond adaptively to challenging situations (action-oriented) (Thompson, 1994). It is thus not surprising that the increasing ability to regulate emotions in childhood is associated with adaptive outcomes in multiple domains, including school readiness (Blair & Razza, 2007), better social skills (Eisenberg et al., 2010), and fewer externalizing problems (Olson et al., 2005).

Emotion regulation manifests in multiple biological, cognitive, and behavioral systems and includes amongst others physiological changes and behavioral responses (Tracy, 2014). Emotions serve a signaling function in which they highlight events as relevant or irrelevant to an individual and help to identify which situations are attention-compelling (and which are not). This signaling function can be assessed by measuring the physiological arousal response, also known as emotional reactivity or affective arousal (Gross, 2013). Events that are signaled as relevant (e.g., a barking dog) will activate the autonomic nervous system (Sapolsky, 2004), including the sympathetic nervous system (SNS) that stimulates increased respiratory rate and heart rate and prepares the body both physiologically and behaviorally to act (e.g., run away or freeze; Porges & Furman, 2011). Having sufficient emotional reactivity (SNS activity) is related to greater self-soothing, more attentional control, and greater capacity for social engagement (Blair & Peters, 2003; Calkins et al., 2002; Calkins & Keane, 2004). On the other hand, inadequate emotional reactivity has been linked to both childhood externalizing and internalizing behavior problems (Beauchaine, 2001; Boyce et al., 2001), linking physiological arousal to psychopathology.

By signaling the demands of the environment, emotional reactivity enables the coordination of behavioral responses that facilitates adaptive behavior (Gross, 2013). Behavioral responses can include the expression of emotions as well as the enactment of regulation strategies. The first, expression of emotions, serves an important social and communicative function (Greenberg, 2004). The display of (facial) emotions can elicit behavior in others which subsequently influences the ongoing interaction. For example, showing fear can elicit others to approach for help, whereas showing anger can signal others to avoid and withdraw (Marsh et al., 2005). In young children, the frequency and intensity of emotional expressivity has been linked to the quality of social relationships (Diaz et al., 2017; Eisenberg et al., 1993) and the child's feelings of social competence (Waiden & Field, 1990). Individual differences in the expression of negative emotions also relate to externalizing problem behavior in typical developing children (Eisenberg et al., 2001), highlighting that the amount and intensity of emotion expressivity will likely have differential effects on person-situation interactions and thus influence social and emotional functioning.

In addition to emotion expressivity, emotion regulation strategies also facilitates adaptive social and emotional functioning. The availability and the variety of emotion regulation strategies is essential to adequately influence the occurrence of emotions and to cope with the intensity, duration, and expression of emotions (Gross, 2013): it is a sign of psychological flexibility when someone is able to choose from different behavioral options in order to cope with a challenging situation. However, it is not only the expression of emotion nor behavioral opportunities that aid self-regulation. For adequate psychosocial functioning, a concordant system of matching emotional internal and external processes is key. When the overt display of emotions matches the internal arousal response (e.g., emotional concordance), it informs the environment on the internal state of the child which enables others to adequately responds to a child's needs (Robinson et al., 1997). Discordance however can significantly hinder the engagement of the environment and confuse others about actual internal states (Mauss et al., 2011) provided that the decision of a caregiver to engage or retreat from interaction with the child depends on the child's display of emotion

(Denham, 1998). Thus, emotional expressivity and the concordance with the physiological arousal response is of key importance in terms of adaptive social and communicative functioning and relevant when studying neurocognitive aspects of self-regulation.

Studies so far showed that adolescents and adults with SCT can have difficulties in multiple areas of emotion regulation, including physiological reactivity and behavioral responses. For example, emotional reactivity (expressed in skin conductance levels) was overall increased in adult men with 47,XXY in response to emotion evoking social situations on video (van Rijn, Barendse, et al., 2014). This is in line with self-reported experience of emotion, where men with 47,XXY typically describe themselves as being more easily aroused by emotion-evoking situations than peers (van Rijn et al., 2006). Adult men with 47,XXY also report more use of atypical behavioral strategies, including increased expression of emotions, avoiding, distraction seeking, and passive regulation (van Rijn & Swaab, 2020). Furthermore, the effect of emotion dysregulation on daily life is also present: emotional outbursts (Visootsak & Graham Jr, 2009), anxiety symptoms (van Rijn, Stockmann, et al., 2014), and depressive symptoms (Tartaglia et al., 2010) are commonly present in SCT in the full age range from school-aged children to adulthood. Studies on emotions in individuals with SCT mainly included behavior (self-reported) questionnaire data and only two other studies so far have examined direct psychophysiological indices (Urbanus et al., n.d.; van Rijn, Barendse, et al., 2014). Furthermore, concordance between emotional constructs has not yet been studied in individuals with SCT, let alone in early childhood.

The Importance of Examining Self-Regulation in Early Childhood

Previous studies that examined self-regulation in the SCT population included school-aged children, adolescents, and adults. There have been very limited systematic studies on neurocognition (including self-regulation) in early childhood, specifically before 6 years of age. However, the preschool period (the period between 3 and 6 years of age) is of particular interest when it comes to self-regulation, given that several aspects that contribute to good self-regulation develop at an accelerated pace in the preschool years (Blair & Ursache, 2011; Zelazo et al., 2008).

This acceleration is partly due to increased connectivity between neural networks in the brain within this period (Posner & Rothbart, 2000), as well as changes at the contextual level (social experience (Carlson, 2005)) and other cognitive abilities (increasing memory capacity, increasing language abilities and accelerated information processing (Hale, 1990)). Additional processes that support this development are increased physical and behavioral skills. As such, self-regulation in infancy and early childhood can be seen as a crucial developmental milestone to be achieved (Blair & Ursache, 2011).

Studying this important window in child development in individuals with SCT may help to understand the impact of an extra X or Y chromosome on the developing brain. Studies that examine typical brain development in childhood show clear indications that the childhood brain is extremely plastic (Andersen, 2003), suggesting that children might be more susceptible for intervention during this period of time (so-called window of opportunity). Examining how neurocognitive skills emerge during this time period could reveal disturbances in brain maturation in children with SCT that are indicative of an at-risk development. The earliest ground work of neuropsychology from A.R. Luria (1963) proceeds this notion, considering that the various stages of mental development encountered as children mature can be seen as a unique opportunity to study how neurocognitive processes develop (Horton, 1987). Thus, information on how children (at risk) develop could guide future research and clinicians in developing and implementing early preventive, neurocognition targeted interventions in this population, in order to minimize the impact of the extra sex chromosome on development.

Clinical Implications

The study of early development of children with SCT becomes more relevant every day, especially given that the number of prenatally identified sex chromosomal aneuploidies is increasing exponentially (Howard-Bath et al., 2018). Amongst other things, this increase stems from technological advances in prenatal testing that led to the introduction of noninvasive testing (NIPT): a screening that isolates cell-free fetal DNA in maternal blood to detect chromosomal aneuploidies

during pregnancy (Carlson & Vora, 2017). In more than 60 countries, NIPT is available to all mothers, regardless of age or risk level, that request prenatal testing to identify fetal abnormalities (Allyse et al., 2015). Upon disclosure of a genetic condition during prenatal testing, the majority of parents experience a certain amount of stress, most often related to the uncertainty of the child's prognosis or opportunities for early preventive intervention (Dinc & Terzioglu, 2006; Jaramillo et al., 2018). To accurately address questions parents may have and to minimize stress burden of parenting a child with a genetic condition, there is a clinical need for updated knowledge on the development of children with SCT (see Box 1 for two clinical vignettes). This knowledge is not only essential to improve genetic counseling, but also to guide clinical care in terms of early assessment and treatment to minimize the impact of the additional X and Y chromosome on development.

Box 1

Clinical Vignettes of Sex Chromosome Trisomies

A) Questions during prenatal testing. It is Tuesday morning and my first client is an expecting female and her partner, 20 weeks into the pregnancy. Last week, they received the results of their non-invasive prenatal screening: They are expecting a girl with Trisomy X. The screening results will need to be verified with other testing, but the soon to be parents were instructed to consult with the TRIXY center of expertise to be the upmost prepared. Both partners are anxious: what does it all mean? Is their daughter going to be "extra feminine"? Will she have learning difficulties? Can she go to a regular elementary school or will she need additional support? Will she have friends? Will she have children of her own? What can they expect as parents, will it be different raising a child with this syndrome? They have already Googled the syndrome and websites state that many girls with Trisomy X have ADHD, will their daughter have that as well? Is it like Down's syndrome?

B) Clinical assessment and treatment. Parents registered their 5-year-old son Arthur with TRIXY Center of Expertise because of concerns about his cognitive and social-emotional development.

Arthur was diagnosed with XYY-syndrome at the age of 4. From a young age, parents felt that Arthur was developing differently and have sought help and guidance from different professionals, but not satisfactorily. Parents would like to have a clear overview of Arthur's development, given that some aspects of his development seem delayed while other aspects appear age appropriate. They wonder what the relation is with XYY. In addition, suggestions regarding treatment and specific support for Arthur are requested. Furthermore, parents are concerned about Arthur's education, they would like suggestions on whether he can stay at the current school (special education for children with language and communication disorders).

Aims and Outline of this Dissertation

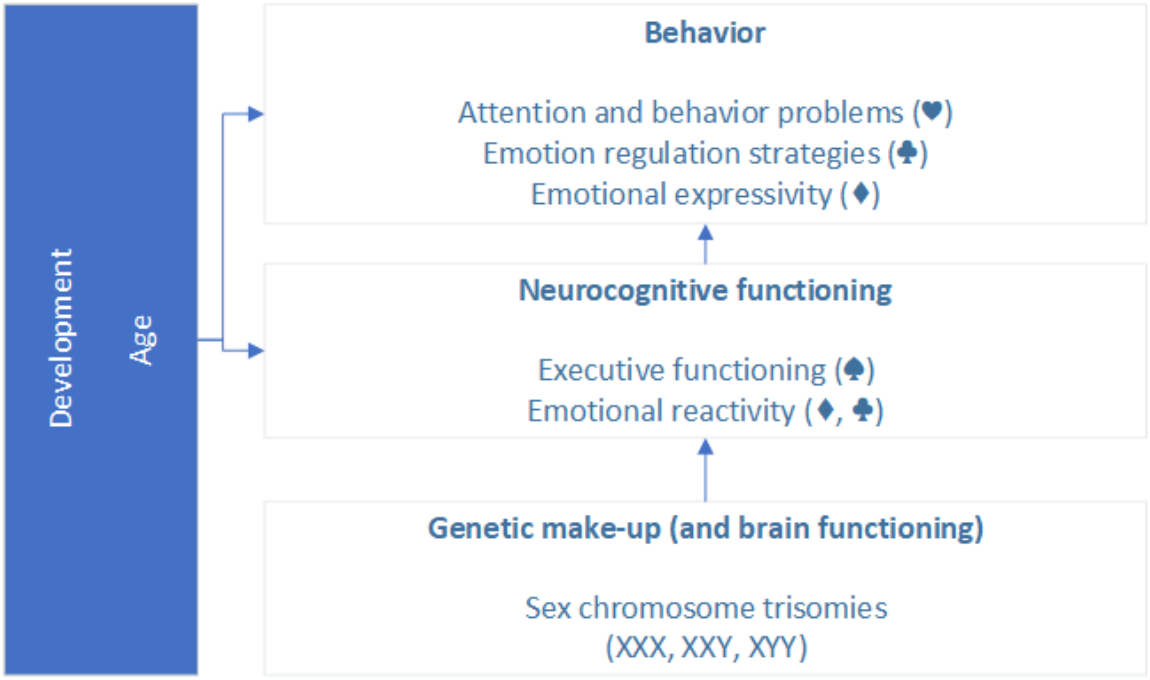
The central aim of the current dissertation is to study neurocognitive aspects of self-regulation in young children with SCT, in terms of behavioral regulation, cognitive regulation, and emotion regulation. The studies presented in this dissertation are part of a larger longitudinal study, the TRIXY Early Childhood Study, and its participants are children with SCT, aged between 1 and 7 years old, recruited from the Netherlands (and surrounding countries) and Denver, Colorado in the United States of America, who are compared to typically developing children from the general population.

Figure 3 provides a visual overview of the neurocognitive domains that were studied in relation to self-regulation in children with SCT in the current dissertation. The first study (**Chapter 2,** ♥) addresses the question whether difficulties with self-regulation are already present in young children with SCT, using parent-reported behavioral data. This study investigates the presence, variety and severity of ADHD symptoms as markers of difficulties in the regulation of thoughts, emotions, attention, behavior, and impulses in daily life behavior (behavioral regulation). **Chapter 3** (♠) focuses on (precursors of) executive functions: the neurocognitive skills essential to act purposefully and show goal-directed behavior (cognitive regulation). By using a combination of cognitive performance data and parental behavioral report data, this study aims to provide insight in

emerging executive functions in young children with SCT and its presentation across different ages. In **Chapter 4** (♦), we examine self-regulation in terms of emotional reactivity and expressivity of children with SCT in response to a laboratory controlled situation designed to induce stress (emotion regulation). Next to physiological arousal (in terms of heart rate), facial and bodily expressions of emotions were coded to study the overlap between the internal experience and the outside display of emotions. In **Chapter 5** (♣), we assess the use and variety of emotion regulation strategies during a frustration-inducing paradigm, whilst controlling for individual reactivity (physiological arousal). In addition, the developmental impact of SCT on emotion regulation strategies is examined here as well. In **Chapter 6**, the results of aforementioned studies are discussed and related to other key neurocognitive domains, including language, communication, and social cognition, that were also assessed during the TRIXY Early Childhood Study. These combined data provide essential insights in the link between neurocognition and psychopathology and pinpoint neurocognitive targets for early, preventive interventions. Finally, **Chapter 7** summarizes the findings, conclusions, and implications of these studies as well as its limitations, and directions for future research are provided.

Figure 3

Visual overview of the neurocognitive domains in relation to early self-regulation skills that were studied in the current dissertation



CHAPTER 2

Early developmental impact of sex chromosome trisomies on Attention Deficit-Hyperactivity Disorder symptomology in young children

Published as:

Kuiper, K., Swaab, H., Tartaglia, N., & van Rijn, S. (2021). Early developmental impact of sex chromosome trisomies on attention deficit-hyperactivity disorder symptomology in young children. *American Journal of Medical Genetics Part A*, 185A: 3664– 3674.

https://doi.org/10.1002/ajmg.a.62418

Abstract

Individuals with sex chromosome trisomies ([SCT], XXX, XXY, and XYY) are at increased risk for neurodevelopmental problems, given that a significant portion of the sex chromosome genes impact brain functioning. An elevated risk for psychopathology has also been described, including Attention Deficit-Hyperactivity Disorder (ADHD). The present study aimed at identifying early markers of ADHD, providing the first investigation of ADHD symptomology in very young children with SCT. The variety, type, and severity of ADHD symptomology in 1-to-6-year-old children with SCT (*n*=104) were compared with population-based controls (*n*=101) using the Strengths and Weaknesses of ADHD symptoms and Normal-behavior (SWAN) parent-report questionnaire. ADHD symptomology was significantly more prevalent in SCT and already present from toddlerhood on, compared to controls. ADHD inattention symptoms were significantly increased in all karyotypes (XXX, XXY, XYY), boys with XYY also showed significantly more hyperactivity/impulsivity symptoms than controls. Inattentiveness was more pronounced with increasing age for SCT, in contrast to controls. Within the SCT group, 24% of the children had significantly elevated ADHD symptoms at a clinical level. Already from an early age on, SCT is associated with a risk for ADHD, suggesting that its neurodevelopmental risk lies anchored in early brain maturation. Studying this genetically vulnerable population allows for the prospective study of risk markers to facilitate early and preventive interventions.

Early Developmental Impact of Sex Chromosome Trisomies on Attention Deficit-Hyperactivity Disorder Symptomology in Young Children

Sex chromosome trisomies (SCT) are among the most common chromosomal aneuploidies in humans (Hong & Reiss, 2014), with an estimated prevalence around 1 in 650 to 1,000 live births (Berglund et al., 2019; Bojesen et al., 2003; Groth et al., 2013; J. K. Morris et al., 2008). Karyotypes that result from SCT are 47,XXY (Klinefelter's syndrome) and 47,XYY (XYY syndrome) in males, and 47,XXX (Trisomy X syndrome) in females. Many individuals with SCT experience a significant delay in diagnosis or even non-diagnosis throughout life: estimates of non-diagnosis for all three trisomies range from 12% to 25% (Berglund et al., 2019; Bojesen et al., 2003). Clinically, SCT is characterized by mild, variable, and mostly non-specific physical features, including minimal facial dysmorphisms, tall stature, and abnormal muscle tone (hypotonia)(Tartaglia et al., 2020). The clinical presentation of SCT is considered to be diverse and heterogeneity in behavioral and cognitive outcomes is rather rule than exception (Giltay & Maiburg, 2010; Groth et al., 2013; Tartaglia et al., 2010). Whilst knowledge of the somatic phenotype of SCT is amply available, more research on specific domains of the neurocognitive and neurobehavioral profile is needed (Pieters et al., 2011). This is especially important considering that a significant fraction of genes on the X chromosome have been linked to brain functioning and X-chromosome genes are nearly six times more likely to be involved in cognitive performance than genes on the autosomes (Zechner et al., 2001).

Interestingly, neuroimaging studies in individuals with SCT have shown that the X and Y chromosomes impact brain circuits involved in self-regulation (Hong & Reiss, 2014), which refers to regulation of thoughts, emotions, attention, behavior, and impulses in order to meet goals and adequately respond to the environment (Blair & Diamond, 2008). Such self-regulatory skills are of great importance with regards to day to day functioning and quality of life, given that optimal self-regulation promotes positive adjustment and adaptation, as reflected in positive relationships, productivity, achievement, and a positive sense of self (Blair & Diamond, 2008). From a developmental perspective, studies have shown that self-regulation is associated with important

long-term outcomes such as mental health (Moffitt et al., 2011), social competence (Bradley & Corwyn, 2013, 2007), and academic achievement (Eisenberg et al., 2010; Vazsonyi & Huang, 2010), showing that self-regulation is a vital skill to be acquired in child development. Difficulty with self-regulation is in line with the types of symptoms of psychopathology that have been described in SCT (van Rijn, 2019), such as autism spectrum disorder (ASD), mood disorders, but especially attention-deficit/hyperactivity disorder (ADHD).

ADHD is a neurodevelopmental disorder, which is currently defined by a collection of persistent and impaired cognitive and behavioral symptoms, notably inattention, hyperactivity, and impulsivity (DSM-5: APA, 2013). Self-regulation is critical for individuals with ADHD who are challenged in modulating their feelings, thoughts, and responses. With an overall prevalence of 7.2% of ADHD in the general population (Elsabbagh et al., 2012), significantly elevated clinical levels of ADHD symptoms are reported in SCT in several studies across all three karyotypes (van Rijn, 2019). Using dimensional measures across several studies, average estimates of clinical levels of ADHD symptoms are 35% for 47,XXY (with a range of 27-42%); 49% for 47,XXX (with a range of 27 52%); and 69% for 47,XYY (with a range of 62-76%). When considering the DSM classification, on average 43% of 47,XXY (with a range of 24-63%), 49% of 47,XXX (with a range of 25-49%), and 36% of 47,XYY (with a range of 11-52%) meet full diagnostic criteria for ADHD. Although the presentation of ADHD-related symptoms is similarly variable between individuals with SCT, inattentive symptoms are typically the most common in 47,XXY and 47,XXX, whereas 47,XYY boys are likely to present hyperactive/impulsive symptoms as well (Tartaglia et al., 2012).

Previous studies on ADHD symptomology in SCT focused on populations with broad age-ranges, including participants from middle childhood to adulthood. However, information on early development before the age of six years is extremely limited (Urbanus, van Rijn, and Swaab, 2020). This is unfortunate, given that this period in child development is marked by significant advances in brain maturation in typically developing children (Hensch, 2004), making it worthwhile to examine the developmental impact of brain maturation on behavior in a genetically-at-risk population.

Furthermore, because genetic conditions such as SCT can be identified very early in development (already prenatally), before any clinical behavioral presentation, studying young children with SCT may help to understand the early developmental factors that co-determine neurobehavioral pathways. This will provide further insights in addition to what we have learned from studying children with psychopathology according to diagnostic criteria based on behavioral presentation. The present study was designed to investigate the developmental impact of SCT on regulation of thoughts, emotions, attention, behavior, and impulses, as expressed in symptoms of ADHD. Rather than considering ADHD as an all-or-none phenomenon, the present study was targeted at examining the variation in ADHD symptoms, which may provide a more sensitive measure of early regulation deficiencies in young children.

Taken together, the aim of the present study was to evaluate the developmental impact of SCT on ADHD symptoms, with regards to variety, type, and severity, in an international, sample of young children (1-6 years old), compared to population-based control sample. To our knowledge, the current study is the first to investigate the neurodevelopmental risks in terms of ADHD symptoms of young children with SCT during toddlerhood and the preschool period. Comparing age-related differences in a large sample of predominantly prenatally diagnosed SCT children with control peers could provide prospective insight into the early impact of SCT on self-regulation in the developing young brain. Furthermore, by identifying the recruitment strategy, our study also allowed for an empirical investigation of phenotypic differences within the SCT group. Due to advances in non-invasive prenatal testing technology, it is expected that the number of prenatal diagnoses of SCT will substantially increase over the coming years (Tartaglia et al., 2020). Knowledge on the early development is therefore also greatly needed to guide genetic counseling and improve clinical care.

Methods

Participants

The current study is part of a larger international longitudinal study (the TRIXY Early Childhood Study, at Leiden University in the Netherlands, including research sites in the Netherlands and the United States of America [USA]). The TRIXY Early Childhood Study investigates the social, emotional, and behavioral development of young children with a trisomy of the X/Y chromosomes (TRIXY). For the current study, children aged 1 up to and including 6 years (at baseline) were included.

In total, 104 children with SCT with a mean age of 43.85 months (SD = 22.57, range 11-86 months) and 101 population-based controls (control group = CG) with a mean age of 43.30 months (SD = 19.50, range 12-77 months) participated with their primary caregiver. Parental education of the primary caregiver, assessed using the Hollingshead ratings of educational attainment, showed that most of the primary caregivers had at least a post high school degree or training (SCT: MD=5.95, SD=1.16, CG: MD=5.61, SD=1.39). The SCT and control groups did not differ significantly with regards to age ($t(203) = .186$, $p = .852$) nor parental education ($t(203) = 1.891$, $p = .060$). However, group differences existed with regards to gender distribution (i.e., as expected the control group included significantly more girls than the SCT group: $X^2(1, N = 205) = 12.698$, $p < .001$). As for the timing of SCT diagnosis, 71 children (68.3%) had a prenatal diagnosis (i.e., because of [routine] prenatal screening, abnormal ultrasound findings, or advanced maternal age) versus 33 children (31.7%) with a postnatal diagnosis (i.e., because of developmental delay, physical and/or growth problems, or medical concerns). More than half of children with XXY did not receive testosterone replacement therapy (51.0%, $n = 25$) at any given time in their development. With regards to ADHD-diagnosis in the family, parental reports showed that in the SCT group 13 parents (12.5%) and 7 siblings (6.7%) had a diagnosis of ADHD.

Children with SCT were recruited from two sites: first, the Trisomy of the X and Y chromosomes (TRIXY) Center of Expertise at Leiden University in the Netherlands ($n = 46$) that

recruited children from all Dutch-speaking countries in Western Europe, and second, the eXtraordinarY Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado in Denver, USA (*n* = 58) that recruited children from across the USA. Recruitment of children with SCT took place with the help of clinical genetics departments, pediatricians, and national advocacy or support groups for (parents of) individuals with SCT with recruitment flyers and postings on the internet (e.g., TRIXY website and the eXtraordinarY Kids Facebook page). For the SCT group, ascertainment bias was recorded and three subgroups were identified: a) 'active prospective follow-up' (51.0% of the SCT group), b) 'information seeking parents' (29.8% of the SCT group), and c) 'clinically referred cases' (19.2% of the SCT group). Control participants were recruited from elementary schools and daycare centers from the western part of the Netherlands.

All participants were Dutch- or English-speaking (child and parent) and without history of traumatic brain injury, severely impaired hearing or sight, or colorblindness. For children in the SCT group, trisomy in at least 80% of the cells was confirmed by standard karyotyping. Researchers requested parents to present a copy of the karyotyping report of the child, that was provided by their clinician at time of diagnosis. Karyotyping of the child was done by clinical genetic departments, based on the appropriate guidelines for chromosomal karyotyping. The controls were not subjected to genetic screening, due to ethical reasons. These children were considered a representation of the general population and given the prevalence of SCT is ~1 in 1000, the risk of having one or more children with undiagnosed SCT in the control group was considered minimal and acceptable.

Ethics and Procedure

This study was approved by the Medical Research and Ethical Committee of Leiden University Medical Center in the Netherlands and the Colorado Multiple Institutional Review Board (COMIRB) in the USA. Researchers from Leiden University were responsible for project and data-management (i.e., training and supervision of researchers, processing, and scoring of data). Written informed consent was obtained from all parents/guardians. The primary caregiving parent (92%

mother) of the child completed the questionnaires, either in Dutch or in English, using the online survey software Qualtrics (http://www.qualtrics.com/).

Instruments

ADHD Symptoms

The Strengths and Weaknesses of ADHD symptoms and Normal-behavior (SWAN) was selected as a screening tool for ADHD symptomology. The SWAN is a parent-report questionnaire designed to reflect the entire range of attention skills in both nonclinical as clinical populations (Swanson et al., 2012). The SWAN rating scale provides a continuous distribution of both positive and negative evaluations of attention behaviors (Polderman et al., 2007; Swanson et al., 2012), by using a 7-point scale anchored to average behavior (i.e., Far Below Average = 3, Below Average = 2, Somewhat Below Average = 1, Average = 0, Somewhat Above Average = −1, Above Average = −2, and Far Above Average = −3). The questionnaire consists of 18 items that reflect the 18 DSM-5 ADHD symptoms, divided into two subscales of nine items corresponding to the domains of Inattention (items 1-9) and Hyperactivity/Impulsivity (items 10-18). Positive scores indicate parental report of experienced difficulty in attentional skill above average, whereas negative scores indicate better skills than average. The mean of all eighteen item scores results in the Combined scale score and there are also mean total subscale scores on the Inattention items and the nine Hyperactive/Impulsive items. The scales reportedly show good internal consistency, validity, and reliability in different international samples and studies (Polderman et al., 2007; Swanson et al., 2012, 2017). In the current study, the internal consistency coefficients were .91 (Combined), .87 (Inattention), and .87 (Hyperactivity/Impulsivity) which indicate good to excellent internal validity.

Statistical Analyses

Raw Scores

For analyses, raw scores on the SWAN were used to compare SCT and CG children. Furthermore, to assess for clinical risk, a cut-off score was used. The cut-off scores for the SWAN subscales were calculated following guidelines of Swanson et al. (2012), the developers of the

SWAN, by using the mean + 1.65 SD (in z-scores) from the control sample as cut-off. This method has been verified across studies with differential methodologies and proven useful in identifying the abnormal prevalence of ADHD symptoms in 4% of the population (for review: see Brites et al. (2015). In the current study, cut-off scores were .52 (Inattention subscale), and .79 (Hyperactivity/Impulsivity subscale) and resulted in either "below" or "at-risk" category. Also, because the items on the SWAN contain the exact 18 DSM-5 diagnostic criteria for ADHD, participants could be categorized into one of the DSM ADHD subtypes. This was done by following the steps taken by Tartaglia, Cordeiro, Howell, Wilson, & Janusz (2010), who used the predecessor of the SWAN in a similar sample. Participants were determined to meet criteria for ADHD if they were noted to have moderate to severe symptoms in 6 of the 9 inattentive items (ADHD-Inattentive subtype), or in 6 of the 9 hyperactive/impulsive items (ADHD-Hyperactive Impulsive subtype), or in 6 of the 9 items in both inattentive and hyperactive/impulsive domains (ADHD-Combined subtype).

Age Groups

Participants were divided into three groups based on their age: a) 1-2-year-old group (ranging 11-35 months), b) 3-4-year-old group (ranging 36-59 months), and c) 5-6-year-old group (ranging 60-86 months).

Analyses

Statistical Package for the Social Sciences (SPSS) version 25 was used for statistical analyses. General group comparisons were performed using independent sample *t*-tests. Correlations between age and ADHD symptoms within research groups were investigated using Pearson's correlation analysis. Group differences in ADHD symptoms were examined using univariate analysis of variance (ANOVA), within each of the specific age-group, to investigate developmental trajectory. In addressing the relation between age and ADHD symptoms, correlational analyses within the SCT were performed without IQ as a covariate. This approach was based on the work of Dennis et al. (2009) who have argued that correcting for IQ may obscure developmental vulnerabilities that are due to shared processes in terms of overall brain development, resulting in type 2 errors (false

negatives). Effect sizes for *t*-test analyses were calculated with Cohen's *d*, with .2 being a small, .5 being a medium and .8 being a large effect (Cohen, 1977). Level of significance was set at $p \leq .05$, two-tailed. After analyses were completed, participants with extreme outliers on one of the three SWAN variables (Z>3) were identified and all analyses were rerun without these participants to assess their influence on the results. When applicable, the influence of the outliers is described in the results section.

Results

ADHD Symptomology

Table 1 shows the mean scores of the SCT group and control group on the SWAN rating scale. Independent samples t-tests were used to test the differences in mean scores on the SWAN (sub)scales between groups. Children with SCT had significantly more ADHD symptoms in general and specifically more inattentive ADHD symptoms than controls, with medium effect sizes (see Table 1). For the hyperactive/impulsive ADHD symptoms, there was no significant difference in mean scores between children with SCT and controls.

Table 1

Means, SDs, and T-Test Statistics for SCT and CG of ADHD Symptoms

	SCT	CG			
	(*N*=104)	(*N*=101)	Group differences		
	M ± *SD*	*M* ± *SD*	*t*	*p*	Cohen's *d*
SWAN-Combined	.03 ± .68	-.30 ± .52	3.95	<.001	.6
SWAN-Inattention	.09 ± .72	-.45 ± .59	5.78	<.001	.8
SWAN-Hyperactive/Impulsive	-.02 ± .75	-.15 ± .57	1.46	.146	.2

Note. SWAN: Strengths and Weaknesses of ADHD symptoms and Normal-behavior; SCT: sex chromosome trisomies, CG: control group. Negative means represent scores above average on the SWAN, as impairment is rated as more positive.

Karyotypes

To examine whether these increased ADHD symptoms were present in all SCT karyotypes, separate independent samples t-tests were performed for each karyotype to test for mean differences on the SWAN (sub)scales. Children with SCT were compared to their control peers matched on gender (XXX vs. XX, XXY vs XY, and XYY vs XY). Descriptives and *t*-test statistics are given in Table 2.

For girls with XXX, parents reported significantly more ADHD symptoms in general and specifically more inattentive ADHD symptoms compared to control girls, with medium to large effect sizes. There was no significant difference with the control girls for hyperactive/impulsive ADHD symptoms (p = .211). For boys with XXY, parents also reported significantly more inattentive ADHD symptoms compared to control boys, with a medium effect size. There were no significant differences with the control boys for hyperactive/impulsive ADHD symptoms (t = -1.248, p = .215) and total ADHD symptoms (t = .746, p = .458). Finally, for boys with XYY, parents reported significantly more ADHD symptoms in general compared to control boys, with difficulties in both the inattention domain as the hyperactive/impulsive domain, with large effect sizes. Thus, increased ADHD symptoms were found in all karyotypes with difficulties primarily in the inattention domain. In the XYY group, the ADHD symptoms were more pronounced and included problems in the hyperactive/impulsive domain, in addition to inattention difficulties.

Age-Related Effects

Given the finding that increased ADHD symptoms were present in all children with SCT with only minor karyotype specific differences and the fact that all ages were represented equally across karyotypes, we were able to investigate the impact of age on ADHD symptoms in the total SCT group, above and beyond karyotype. To examine the developmental trajectory of ADHD symptoms in children with SCT and controls, separate correlation analyses were performed within the SCT and control groups between age and the three SWAN (sub)scales. The results showed a significant

Table 2

ADHD Symptoms Between Groups: Karyotype-Specific Comparisons

	XXX (*N*=33)	XX (*N*=57)			XXY (*N*=49)	XY (*N*=44)			XYY (*N*=22)	XY (*N*=44)		
	M ± *SD*	*M* ± *SD*	*p*	*d*	*M* ± *SD*	*M* ± *SD*	*p*	*d*	*M* ± *SD*	*M* ± *SD*	*p*	*d*
SWAN Combined	.09 ± .65	-.33 ± .56	<.01	.7	-.18 ± .60	-.26 ± .46	n.s.	-	.41 ± .72	-.26 ± .46	<.001	1.1
SWAN Inattention	.21 ± .74	-.46 ± .63	<.001	1.0	-.11 ± .63	-.43 ± .53	<.01	.5	.33 ± .78	-.43 ± .53	<.001	1.1
SWAN Hyperactive/Impulsivity	-.03 ± .70	-.20 ± .61	n.s.	-	-.24 ± .67	-.09 ±.52	n.s.	-	.50 ± .75	-.09 ± .52	<.001	.9

Note. SWAN = Strengths and Weaknesses of ADHD symptoms and Normal behavior. Negative means represent scores above average on the SWAN, as impairment is rated as more positive.

correlation between age and inattentive ADHD symptoms in the SCT group (r = .234, p < .02), whereas no such relationship existed in the control group (r = .022, p = .824). Using the Fisher r-to-z transformation, the significance of the difference between the two correlation coefficients was tested and yielded a borderline significant difference in strength of the correlation (z = 1.53, one-tailed p = .063). Furthermore, in both groups there was no significant relationship between age and total ADHD symptoms (SCT: r = .158, p = .109, CG: r = -.040, p = .681), and age and hyperactive/impulsive ADHD symptoms (SCT: r = .060, p = .519, CG: r = -.098, p = .331). In other words, inattentive ADHD symptoms increased with age for children with SCT, whilst for controls ADHD symptoms were not related to age and appeared to present relatively similar across ages. To further identify which specific age groups may drive differences between SCT and controls in terms of cross-sectional age trajectory, participants were divided into three age-groups (1-2 years, 3-4 years, 5-6 years) and separate post-hoc ANOVAs were performed within each age-group with ADHD symptoms (SWAN Combined scale, SWAN Inattention subscale, SWAN Hyperactive/Impulsive subscale) as dependent variables and research group (SCT vs CG) as independent variable. Table 3 show the descriptive statistics for all ANOVAs (also see Figure 1).

In the 1-2-year-old age group, univariate ANOVAs for the SWAN (sub)scales indicated significant differences between SCT and controls for inattentive ADHD symptoms only, with a large effect size. No significant group differences between SCT and controls were found for total ADHD symptoms nor hyperactive/impulsive ADHD symptoms. In other words, 1-2-year-olds with SCT did not show increased hyperactivity or impulsivity but showed more inattentiveness as compared to controls. In the 3-4-year-old and 5-6-year-old groups, univariate ANOVAs for the SWAN (sub)scales indicated significant differences for total ADHD symptoms and inattentive ADHD symptoms, with medium effect sizes in the younger group and large effect sizes in the older group. No significant group differences were found for hyperactive/impulsive ADHD symptoms, indicating that in 3-6-year-olds, children with SCT show similar amounts compared to controls. In other words, the 3-6-

Table 3

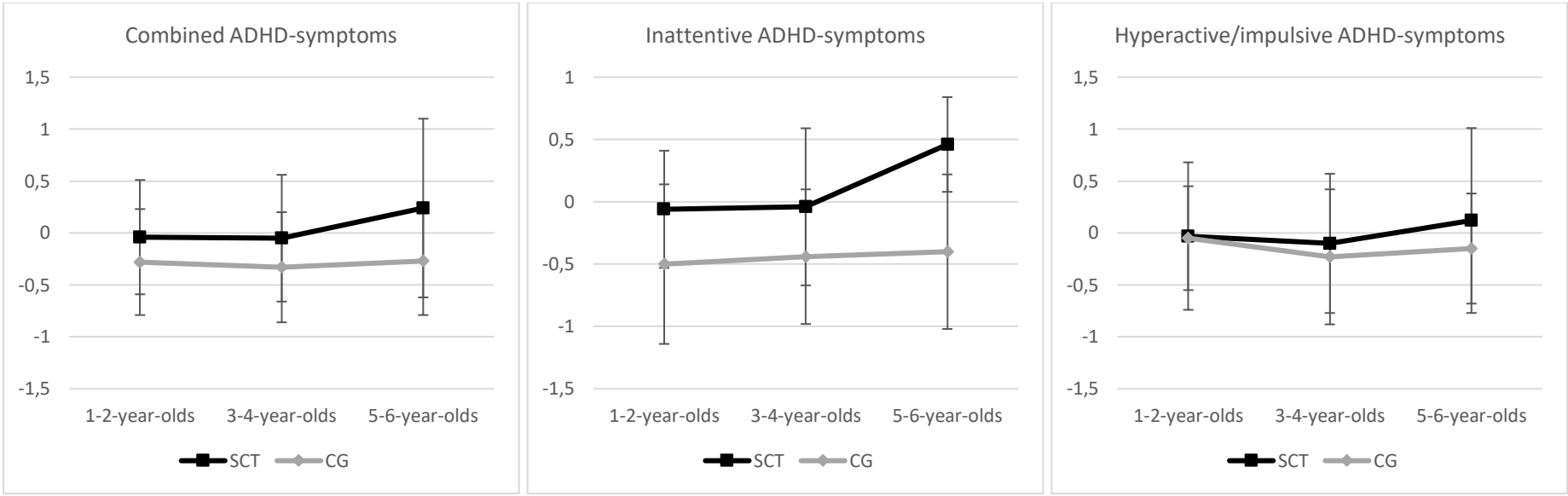
ADHD Symptoms Across Age-Groups

	1-2-year-olds				3-4-year-olds				5-6-year-olds			
	SCT (*N*=35)	CG (*N*=31)			SCT (*N*=40)	CG (*N*=43)			SCT (*N*=29)	CG (*N*=27)		
	M ± *SD*	*M* ± *SD*	*p*	*d*	*M* ± *SD*	*M* ± *SD*	*p*	*d*	*M* ± *SD*	*M* ± *SD*	*p*	*d*
SWAN Combined	-.04 ± .55	-.28 ± .51	n.s.	-	-.05 ± .61	-.33 ± .53	<.05*	.5	.24 ± .86	-.27 ± .52	<.02*	.7
SWAN Inattention	-.06 ± .47	-.50 ± .64	<.01*	.8	-.01 ± .63	-.44 ± .54	<.01*	.7	.38 ± .98	-.40 ± .62	<.01*	1.0
SWAN Hyperactivity/Impulsivity	-.03 ± .71	-.05 ± .50	n.s.	-	-.09 ± .67	-.23 ± .65	n.s.	-	.09 ± .89	-.15 ± .53	n.s.	-

Note. SWAN: Strengths and Weaknesses of ADHD symptoms and Normal behavior. SCT: Sex Chromosome Trisomies. CG: Control Group. Negative means represent scores above average on the SWAN, as impairment is rated as more positive. * Significant at $p < .05$. Effect sizes displayed in Cohen's *d.*

Figure 1

Mean Scores for ADHD-Symptomatology at Different Ages: Sex Chromosome Trisomies (SCT) vs Control Group (CG)



year-old children with SCT did not show increased hyperactivity or impulsivity compared to controls but showed more ADHD symptoms in general and more inattentive behaviors.

Clinical Risk

In addition to average outcomes, we were also interested how many of the children with SCT had scores above clinical cut-off, indicating the severity of ADHD symptoms and the increased risk for ADHD symptomatology. Based on the cut-off score (calculated by taking the Z + 1.65 SD in the CG of each subscale), the number of children with SCT above cut-off were divided by the total number of SCT participants. Results indicated that 24.0% of the children with SCT showed significant inattentive ADHD symptoms, along with 10.6% of the SCT children having hyperactive and impulsive ADHD symptoms above clinical cut-off.

A further examination of this group of children revealed that most of the children were older than 5 years. Because the SWAN questionnaire contains the 18 DSM-5 diagnostic criteria for ADHD, it was also possible to categorize these children, based on the parental report scores, into one of three subtypes of ADHD (similar to the DSM-5 subtypes): ADHD-inattentive subtype, ADHD-hyperactive/impulsive subtype, and ADHD-combined subtype. A fourth category was included that represented no ADHD. For this sub-analysis, we examined only those children with SCT of 5 years and older. Almost half (44.8%) of the 5-6-year-old children met behavioral criteria of ADHD, with 31.0% predominantly inattentive symptoms and 13.8% presenting both inattentive and hyperactive/impulsive symptoms (combined).

Additional Analyses

Ascertainment Bias

To examine whether ascertainment bias was relevant to the increased risk for ADHD symptoms, three separate between-subjects ANOVAs were performed with ADHD symptoms (SWAN Combined, Inattention, and Hyperactivity/Impulsivity (sub)scales) as dependent variables and ascertainment bias within the SCT group (prospective follow-up, information seeking parents, clinically referred cases) as independent variable. Because age did not significantly differ between

the three groups, it was not included as a covariate in the analysis. There were no significant differences in degree of ADHD symptoms (Pillai's trace=.052, $F(6,200)=.884$, $p=.508$): how children enrolled in the study did not appear to affect the degree of ADHD symptoms (also see Table 4).

Table 4

Differences in ADHD Symptoms: Ascertainment Bias Within the SCT Group

	Prospective follow-up (N = 53) $M \pm SD$	Information seeking parents (N = 31) $M \pm SD$	Clinically referred cases (N = 20) $M \pm SD$	p
SWAN Combined	-.03 ± .73	-.03 ± .57	.30 ± .68	.142
SWAN Inattention	.01 ± .78	.04 ± .51	.37 ± .78	.137
SWAN Hyperactivity/Impulsivity	-.06 ± .75	-.10 ± .75	.23 ± .74	.248

Note. SWAN: Strengths and Weaknesses of ADHD symptoms and Normal-behavior, SCT: Sex chromosome trisomies. Negative means represent scores above average on the SWAN, as impairment is rated as more positive.

Recruitment Site

To examine whether recruitment site was relevant in the increased risk for ADHD symptoms in children with SCT, three separate between-subjects ANCOVAs were performed with ADHD symptoms (SWAN Combined, Inattention, and Hyperactivity/Impulsivity (sub)scales) as dependent variables and recruitment site (the Netherlands, the United States of America) as independent variable. Because age differed significantly between the two groups (i.e. SCT children from the USA are significantly younger), it was included as a covariate in the analysis as well. There were no significant group differences in ADHD symptoms (Pillai's trace = .005, $F(6,200) = .157$, $p = .925$): the country from which children were recruited and assessed did not appear to affect the degree of

ADHD symptoms. Parenting rating of ADHD symptoms were similar on all three (sub)scales in the two research sites: total ADHD symptoms (NL: *M* = .07, *SD* = .75, USA: *M* = .01, *SD* = .63), inattention ADHD symptoms (NL: *M* = .15, *SD* = .85, USA: *M* = .04, *SD* = .59), and hyperactive/impulsive ADHD symptoms (NL: *M* = -.01, *SD* = .76, USA: *M* = -.03, *SD* = .74).

Discussion

This is one of the first case-controlled studies investigating the early impact of SCT on regulation of thought and behavior as expressed in ADHD symptomology in a large international sample of young children (1-6 years old). Type and severity of ADHD symptoms were measured using a sensitive, well-known, and widely used instrument (the SWAN rating scale), which allows for capturing the full range of attentional behaviors that reflect symptoms of ADHD in daily life, not limited to classification of ADHD as an all-or-none phenomenon. The current study showed that, on average, the level of ADHD symptoms in SCT was higher than in the general population sample, in the full 1-to-6-year age range. More specifically, children with SCT had more behavioral challenges in the domain of inattention reported by their parents, indicating more difficulties with regulating their attention. Furthermore, behaviors associated with ADHD increased with age, more strongly so in the SCT group, although differences with control peers were already evident for the youngest age-group (1-2-year-olds). From a clinical perspective, 24% of the children with SCT had scores in the clinical range on parent-report, indicating significantly elevated levels of ADHD symptoms. Levels of ADHD behaviors were largely similar across karyotypes, although boys with an extra Y chromosome showed more and broader impairments than children with an extra X chromosome. In addition to inattention difficulties, boys with 47,XYY also exhibited difficulties with hyperactivity and impulsivity. Ascertainment bias and country of recruitment were not relevant to the increased risk of ADHD symptoms, underlining the robustness of these findings.

The most notable finding of this study is that the increased risk for ADHD-symptomology previously reported in older children and adults with SCT, was already found in 1-2-year-olds with SCT. Previous studies have shown that attentional difficulties are part of the behavioral profile of

children with SCT, with predominantly inattention behaviors and to a lesser extent hyperactive and impulsive behavior (Ross et al., 2012; Tartaglia et al., 2012). The current study suggests that these attentional difficulties already exist in very young children with SCT, pointing to a significant neurodevelopmental risk from toddlerhood onward. Given the fact that a significant fraction of the genes on the sex chromosomes are involved in brain development, this elevated risk for attentional difficulties may be one of the first signs that the child's genetic makeup has impacted the brain's development and, more specifically, the brain areas that are important for self-regulation. The self-regulation problems in this young SCT group corresponds to what has been described in older children, adolescents, and adults with SCT, in terms of various and diverse symptoms of psychopathology, such as autism spectrum disorder and ADHD. Considering the importance of self-regulation for adaptive functioning, participation in society, and mental health, these early signs of ADHD symptomology may mark 'at risk' developmental pathways within this genetic population. It is important to point out that although differences between children with SCT and controls on average were significant with medium to large effect sizes, only a subgroup of children had scores in the clinical range. Thus, while some parents may already be recognizing some early attentional concerns in their child compared to peers their age, there are also many parents who do not report any or only mild concerns.

Another main finding of this study is that ADHD symptoms were more pronounced with increasing age; in children with SCT, older age was associated with higher levels of inattentive ADHD symptoms, whereas in controls these symptoms presented stable across ages. Looking at the different age-groups, the presence of ADHD symptoms was the most pronounced in the oldest age-group of children (5-to-6-year-olds), with large effect sizes. The result that age was significantly related to attentional self-regulatory difficulties in children with SCT, but not in controls, could reflect increasing problems that may emerge and present more profoundly with age. This relates to what is called the "growing into deficit" phenomenon (Rourke et al., 1983). As a result of neuroanatomical maturation, the functionality of the brain increases which is reflected in behavioral

opportunities and advancing neurocognitive functions in the developing child. The development of neurocognitive functions occurs in a relative stepwise pattern, in which the next step is dependent on the succession of previous steps. Early disturbances of the neuroanatomical growth, for a substantial part driven by genetic make-up, could therefore impact the succession of the upcoming developmental steps. However, the effects of some of these disturbances may only emerge into behavioral difficulties at a later point in time when a developmental task is presented for which the brain is not yet fully equipped. Also, several neurocognitive functions come 'online' at different and later stages of development, due to the maturation process of the brain, making it possible that the effect of early disturbances may only become noticeable many years later in development. Albeit cross-sectionally, our results indicate that self-regulation problems as expressed in ADHD symptoms may emerge with increasing age in children with SCT, which stresses the importance of a developmental perspective on neurobehavioral outcome in individuals with SCT. Longitudinal studies are needed to provide further clarity on the developmental trajectories.

The current findings contribute to a clearer understanding of the behavioral profile of young children with SCT and specifically show that self-regulatory difficulties with regards to attention are part of the variability and heterogeneity of the SCT behavioral profile. Studying children with a genetic disposition that can be diagnosed prenatally provides a unique opportunity to examine developmental genetic-behavioral-pathways, implementing a prospective approach that goes beyond describing problematic behavior and instead focuses on identifying early markers of 'at risk' development, irrespective of outcomes. From a neuropsychological perspective, it is interesting to examine which information processing deficits related to self-regulation might underly the behavioral profile of children with SCT. Neuro-imaging studies consistently show neuroanatomical and functional differences relative to control peers (Hong & Reiss, 2014), addressing the relevant research question if and how underlying neurocognitive functions might relate to the behavioral profile of young children with SCT. Earlier studies (Lee et al., 2011; van Rijn & Swaab, 2015) have already shown that difficulties with executive functions present across the lifespan of individuals

with SCT (e.g., school-aged, adolescents, and adults). Moreover, there is also some evidence that executive dysfunction and self-regulation could be linked, with studies showing associations between impaired executive functions and increasing externalizing behavior problems symptoms of ADHD and ASD (van Rijn & Swaab, 2015). Investigating the early relations between developing neurocognitive functions and the behavioral profile may help in identifying children with SCT who are prone to developing self-regulatory difficulties and may provide targets for early intervention. Research is also needed to investigate whether the attentional difficulties in SCT are a consequence of problems in other domains (e.g. cognitive, social, or emotional deficits) or whether these difficulties represent a broader impairment in regulatory skills in general. This would be interesting given that preliminary results from the same sample of SCT children showed that the behavioral profile of these children is diverse and heterogeneous (Urbanus, Swaab, et al., 2020), suggesting that regulatory difficulties are present and persistent in multiple developmental domains (e.g., social, emotional, and behavioral).

Even though the current study is the first to date that examines the development of a large, international cohort of young children with SCT to well-matched control peers, our findings should be interpreted in light of several limitations. Due to the limited distribution of children with different karyotypes over the separate age-groups, specific questions could not be examined. For example, it might have been interesting to examine whether the development of attentional difficulties across ages is similar for different karyotypes. Furthermore, the current study examined cross-sectional differences with regards to age and attentional behaviors. A longitudinal design is needed to add validity to the developmental outcomes found in this study. Thirdly, although parents were asked to report on any known diagnosis of ADHD in the family, we did not examine the relation between background genes and the vulnerability for ADHD, due to limited sample size and therefore limited power to test this hypothesis. However, now that we have established the increased risk for ADHD in this population, an interesting follow-up question would be whether a part of the increased risk is attributed to a genetic familiar vulnerability. However, this calls for a meticulous designed study of

background loading, in which affected first- and second-degree family members are identified properly and where genetic factors are related to more developmental domains, other than ADHD alone. Lastly, the current study did not examine the effect of early testosterone hormone treatment on the behavioral profile in the SCT subgroup with XXY. Only a randomized and placebo-controlled trial could provide adequate and reliable insight into the effects of testosterone in infants with Klinefelter: one of which is currently underway (PI Davis, NCT03325647).

The results of this study also have important implications for clinical care. Although the focus of this study was to describe the broad attentional profile of children with SCT, rather than considering ADHD as an all-or-nothing clinical phenomenon, and most children with SCT do not have significant problems in this area, a subgroup of children with SCT are at a substantial risk and might meet full diagnostic criteria of ADHD. These results indicate that all professionals working with individuals with SCT should be aware of the broad behavioral profile and provide routine monitoring and screening of (attentional) regulatory difficulties from an early age on. Following clinical standards with regards to ADHD assessment (e.g. diagnostic interviewing, neuropsychological assessment, and collateral information from school and parents), an early recognition of ADHD (symptoms) in children with SCT calls for early intervention and treatment. Specifically neuropsychological assessment could provide useful information on an individual's strengths and weaknesses and his/her accompanying needs. Early intervention is important because our results show that, as compared to children from the general population, ADHD symptoms are found to be more pronounced with increasing age in SCT. No different from children with ADHD without SCT, treatment for children with SCT and a clinical diagnosis of ADHD ought to be multimodal and focused on limiting the impact of the attentional difficulties on development. Above all, psychoeducation and support for parents and (pre)school with frequent follow-ups should be included in the treatment plan. Although pharmaceutical treatment is often considered part of the treatment plan for ADHD and has been reported to be effective for symptom improvement in older children with SCT and ADHD (Tartaglia et al., 2012), careful consideration is needed when deciding on

implementing medication for a child in the preschool age group. It should include balancing the benefits and risk of medication in the important period in brain maturation of these young children. Furthermore, cultural differences in the use of psychostimulants may also apply. Thus, when considering pharmaceutical treatment, parents should seek out consultation and guidance from a licensed psychiatrist or developmental-behavioral pediatrician with experience in complex neurodevelopmental disorders.

Conclusion

To conclude, in this study it was found that young children with sex chromosome trisomies (47,XXX, 47,XXY, and 47,XYY) are at an increased risk for ADHD symptoms, specifically inattentiveness, and that this risk is already present from toddlerhood onwards. The elevated risk is roughly similar across all three karyotypes, with boys with an extra Y chromosome also showing more hyperactive/impulsive symptoms compared to controls. Moreover, the results showed that ADHD symptoms are higher with increasing age in children with SCT, in line with relevant self-regulation skills coming 'on-line' over the course of neurodevelopment, depending on brain maturation. The current findings suggest that self-regulatory skills, as expressed in symptoms of ADHD, are already impaired in young children with SCT, leading to the proposition that neurodevelopmental problems are likely anchored in early brain development of individuals with SCT. Furthermore, these insights give rise to the hypothesis that the differential behavioral problems of this population in later development might be associated to early self-regulatory difficulties. Self-regulation might be a key factor in explaining behavioral difficulties, also because of its importance in typical development. Future studies are necessary to examine neurocognitive measures of self-regulation, given that different information processing deficits could relate to the behavioral problems associated with SCT. Moreover, studies with a longitudinal approach could provide insight into the developmental trajectories of young children with SCT and investigate how self-regulatory skills develop in this population as well as its predictive value over time. Nevertheless, these early signs of self-regulatory deficits might serve as an at-risk marker in SCT, allowing the identification of

children with at-risk development and guide preventive and early interventions optimizing outcomes of these children. From a clinical perspective, clinicians should be aware of the neurodevelopmental risk with regards to self-regulation in children with SCT and monitor the neurodevelopment of these children, given that a significant portion of these children at this young age are already at clinical risk for elevated ADHD symptoms.

CHAPTER 3

The developmental impact of sex chromosome trisomies on emerging executive functions in young children: Evidence from neurocognitive tests and daily life skills

Published as:

Kuiper, K. C., Swaab, H., Tartaglia, N., van Buggenhout, G., Wouters, C., & van Rijn, S. (2022). The developmental impact of sex chromosome trisomies on emerging executive functions in young children: Evidence from neurocognitive tests and daily life skills. *Genes, Brain and Behavior*, 21 (6): e12811. doi:10.1111/gbb.12811

Abstract

Sex chromosomal trisomies (SCT) are associated with impairments in executive functions in school-aged children, adolescents, and adults. However, knowledge on preschool development of executive functions is limited but greatly needed to guide early intervention. The current study examined emerging executive functions in young children with SCT. Participants were 72 SCT children and 70 population-based controls, aged 3 to 7 years, who completed a neurocognitive assessment of both global executive function (MEFS) and verbal executive function skills (NEPSY Word Generation). Caregivers completed the Behavior Rating Inventory of Executive Function (BRIEF) questionnaire to capture real-world behavioral manifestations of impairments in executive functions. Results showed that impairments were significantly more prevalent in SCT than in controls and already present from 3 years, specifically verbal executive functions and working memory. Broader more pronounced impairments were found in older children with SCT. Age was significantly related to executive functions, but specific domains showed different relations with age. For example, deficits in planning and organizing remained evident with older age in SCT whereas it declined with age in controls. Impairments in executive functions were present across different levels of intelligence. Already at an early age, impairments across executive functions should be considered part of the neurodevelopmental profile of SCT, which appear more prominent at later age. Future studies should investigate developmental pathways of executive functions in SCT, given its relevance in cognitive, social, and emotional development. Executive functions should be screened and monitored in children with SCT and could be an important target of preventive intervention.

The Developmental Impact of Sex Chromosome Trisomies on Emerging Executive Functions in Young Children: Evidence from Neurocognitive Tests and Daily Life Skills

With a high prevalence of 1-2 in 1000 births, sex chromosomal trisomies (SCT) are one of the most common chromosomal aneuploidies (Berglund et al., 2019; Groth et al., 2013). Karyotypes that result from SCT are 47,XXY (Klinefelter syndrome) and 47,XYY (XYY syndrome) in males and 47,XXX (Trisomy X syndrome) in females. Recent technological advances allow for safe and earlier screening for genetic syndromes and are expected to lead to an increase of the number of prenatally diagnosed children with SCT (Samango-Sprouse et al., 2017). This calls for more knowledge on the developmental impact of SCT which is needed to improve genetic counselling and clinical care for children with these conditions. Also, studying genetic conditions such as SCT from pregnancy on provides a unique opportunity to prospectively examine early neurocognitive development and its link to later developmental outcome.

Having an extra X or Y chromosome not only impacts physical development but also neurodevelopmental and psychological functioning (Tartaglia et al., 2020). This is not surprising given the high density of genes on the sex chromosomes that are essential for brain development (Zechner et al., 2001), putting children with SCT at increased risk for neurodevelopmental problems (i.e., impairments of growth and development of the brain, that may lead to differences in brain functioning and thus emotion and cognition amongst other domain). So far, neuroimaging studies have shown that brain architecture and functioning appears different in individuals with SCT compared to peers from the general population (XXY: Steinman et al., 2009; XXX/XXY/XYY: Warling et al., 2020). Furthermore, underlying information processing difficulties are also found in individuals with SCT with a quarter of the group showing difficulties of clinical relevance (for a review see van Rijn, 2019). Amongst other domains, impairments are found across executive functions whilst intellectual functioning is usually within the typical range (although at the lower end, particularly for verbal IQ)(XXY and XXX: Lee et al., 2011; XXY: Janusz et al., 2020). Of relevance to the current study are studies showing neuroanatomical and functional differences in the (pre)frontal cortex in

individuals with an extra X chromosome (Itti et al., 2006; Lentini et al., 2013), an area strongly involved in executive functions (Posner & Rothbart, 2006).

The term *executive functions* (EF) refers to a set of interrelated cognitive skills essential to learn, cope, and manage daily life (Diamond, 2013). Executive functions are responsible for purposeful, goal-directed, and problem-solving tasks and behavior. Several components can be identified, including attention, inhibition, monitoring, flexibility, working memory, planning, and fluency (Anderson, 2001). Proper executive functions are crucial when it comes to positive childhood development: executive functions promote mental and physical health; predict success in school and in life; and support cognitive, social, and psychological development (Diamond, 2013). On the other hand, impairments across executive functions are involved in many neurodevelopmental disorders, including attention deficit hyperactivity disorder (ADHD, Diamond, 2005), autism spectrum disorder (ASD, Demetriou et al., 2018), and intellectual disabilities (Lee et al., 2015).

Until now, studies that have examined executive functions in individuals with an extra X or Y chromosome showed, on average, reduced executive function performance compared to controls from the general population (for review see Urbanus et al., 2020). Children with SCT show more impairments across executive functions, including attention, inhibition, mental flexibility, working memory, and planning/problem solving (Janusz et al., 2020; Lee et al., 2015; Ross et al., 2008, 2009; Samango-Sprouse et al., 2018; van Rijn & Swaab, 2015). In daily life, parents of children with an extra X chromosome (XXX and XXY) report difficulties in (sub)domains of behavioral regulation and meta-cognition (Janusz et al., 2020; Lee et al., 2015). A substantial part of the SCT group shows significant executive function difficulties, that are present across studies and assessments. For example, 19% to 57% of children with SCT (both Dutch and American) show a clinical score on sustained attention tasks (Samango-Sprouse et al., 2018). Furthermore, impairments in executive functions in children with SCT have been linked to increased externalizing behavior problems (van Rijn & Swaab, 2015), increased social difficulties (Skakkebæk et al., 2017) as well as increased symptoms of ASD (van Rijn et al., 2012), psychotic symptoms such as disorganized thought (Van Rijn et al., 2009), and ADHD

symptoms (Lee et al., 2011). It is thus not surprising that parents frequently mention that their child's executive dysfunction, amongst others, is a major barrier to learning and academic development (Thompson et al., 2021).

Previous studies that examined executive functions in the SCT population included school-aged children, adolescents, and adults. There have been very limited systematic studies on executive functions in early childhood, specifically before 6 years of age and prior to starting the early school years. However, the preschool period (the period between 3 and 6 years of age) is of particular interest when it comes to executive functions, given its development accelerates in the preschool years (Zelazo et al., 2008). This acceleration is partly due to increased connectivity between neural networks in the brain within this period (Posner & Rothbart, 2000), as well as changes at the contextual level (such as social experience (S. M. Carlson, 2005)) and other cognitive abilities (increasing memory capacity, increasing language abilities and accelerated information processing (Hale, 1990)). Studying this important window in child development in individuals with SCT may help to understand the impact of an extra X or Y chromosome on the developing brain. Differences with typically developing peers are to be expected, given that a high density of genes on the sex chromosomes are essential for brain development (Zechner et al., 2001), putting children with SCT at increased risk for neurodevelopmental problems including impairments across executive functions. Also, early identification of these difficulties may reveal risk markers in the development of children with an extra X or Y chromosome, that could prove helpful in identifying targets for early intervention to improve outcomes later in life.

Assessment of executive functions usually relies on a combination of a direct assessment of information processing skills as well as structured behavioral observations in daily life. There is growing evidence that executive functions represent diverse but also united constructs in early childhood (Collette et al., 2005; Miyake et al., 2000). This has also led to new techniques to measure executive functions in young children, such as the Minnesota Executive Function Scale (MEFS App™) that provides a standardized performance-based assessment of global executive function skills,

designed for children ages 2 and up (S. M. Carlson & Zelazo, 2014). It integrates three basic executive functions (working memory, inhibitory control, and cognitive flexibility) into a single graded scale. Because the assessment is sensitive to age and performance, following an adaptive testing protocol, it provides the opportunity to assess and follow to the development of emerging executive functions; with *emerging* meaning still-developing, not yet stable (Isquith et al., 2004). In addition to the neurocognitive assessment of executive functions, structured observations of behavioral problems in daily life are also crucial. Parents are vital observants in providing information on the behavior of their child to gain insight in the developing functions. To illustrate, a child that has difficulties with cognitive flexibility may experience difficulties with a changing caregiver or shift in routine. Using standardized parental rating systems is a well-accepted evidence-based method in the assessment of social, emotional, and behavioral functioning (Achenbach & Rescorla, 2000). In this study, both neurocognitive tasks and structured observations are used to provide information on executive functions in young children with SCT.

The current study is, to the best of our knowledge, the first to examine emerging executive functions in a large, international cohort of children with SCT between the ages of 3 to 7 years old, compared to population-based controls. As the three trisomy karyotypes (i.e., XXX, XXY, XYY) are characterized by similar neurocognitive impairments during childhood (Urbanus et al., 2020; Kuiper et al., 2021; Bouw et al., 2022), we grouped them into a single sex chromosome trisomy group. The primary goal of the current study was to investigate how executive functions present across different ages in young children with SCT, expressed in terms of information processing skills as well as behavioral observations. Given that executive functions in early childhood are considered a unitary construct, we examined executive functions by using a single performance measure that is appropriate for a large age-span. In addition, a verbal fluency task was used to examine verbal executive functions specifically. This task was chosen as the language domain is an evident vulnerability in children with SCT (Urbanus, van Rijn, et al., 2020) and we wanted to examine emerging executive functions in the context of both verbal and non-verbal based information

processing. Furthermore, the behavioral report allowed for examination of smaller subdomains of executive functions that could inform on specific vulnerabilities of young children with SCT. Based on earlier research with older children and adults, we hypothesized that even pre-school age children with SCT already experience difficulties with executive functions.

Methods

Participants

The current study is part of a large ongoing international longitudinal study (the TRIXY Early Childhood Study, at Leiden University in the Netherlands, including research sites in the Netherlands and the United States of America [USA]). The TRIXY Early Childhood Study investigates the social, emotional, and behavioral development of young children with a trisomy of the X/Y chromosomes (TRIXY). Prior studies from the TRIXY project has been published elsewhere (see for example Bouw et al., 2022; Kuiper et al., 2021). For the current study, children aged 3 up to and including 7 years (at baseline) were included. Children were recruited from two sites: The Trisomy of the X and Y chromosomes (TRIXY) Center of Expertise in the Netherlands that recruited children from all Dutch-speaking countries in Western Europe (n = 39) and the eXtraordinarY Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado (CHC) in Denver that recruited children from across the United States of America (n = 33). The two clinical groups did not differ in terms of gender distribution (χ^2 (1,72)= 1.346, p = .246) nor educational level of caregivers (p = .224), but differed with respect to age with the American SCT group being on average younger than the Dutch SCT group (t(66) = 4.486, p < .001). Children with SCT were recruited with the help of clinical genetics departments, pediatricians, and national advocacy or support groups for (parents of) individuals with SCT by using recruitment flyers and postings on the internet and social media. Three different recruitment strategies were identified for the SCT group (see Table 1): a) ‘information seeking parents’, b) ‘active prospective follow-up’, and c) ‘clinically referred cases’. Children from the control group were recruited from day care centers, public institutions, and elementary schools from the western part of the Netherlands by using recruitment flyers.

In total, 72 children with SCT and 70 age-matched controls from the general population participated in this study with their primary caregiver. The SCT group consisted of 27 girls with 47,XXX, 30 boys with 47,XXY, and 15 boys with 47,XYY. As for the timing of SCT diagnosis, 40 children (56%) had a prenatal diagnosis (i.e., because of [routine] prenatal screening, abnormal ultrasound findings, or advanced maternal age) and 32 children (44%) had a postnatal diagnosis (i.e., because of developmental delay, physical and/or growth problems, or medical concerns). Confirmation of trisomy in at least 80% of the cells was provided by standard karyotyping. Parents were asked to present a copy of the karyotyping report of the child that was provided by their clinician at time of diagnosis. Children from the control group were not subjected to genetic screening. Given the low prevalence of SCT (~1 in 1000) in the general population, we decided that the burn of blood draw for testing for SCT in our control group outweighed their potential utility. We reviewed the possible risk of having a child with undiagnosed SCT in our control group minimal and acceptable. The majority of the children with 47,XXY (57%, *n* = 17) did not receive testosterone replacement therapy at any given time in their development. Parental education level was assessed according to the Hollingshead criteria and ranged from category 1 (no formal education) to 7 (graduate professional training) (Hollingshead, 1975). When the child was raised by two parents (95%), educational level was averaged over both parents. Parental education level varied from 4 to 7 (median 6) in the SCT group and from 2 to 7 (median 5) in control group. All participants were Dutch- or English-speaking. Children had no history of traumatic brain injury, severely impaired hearing or sight, or colorblindness.

To examine the developmental impact of SCT, children were divided into two age groups: 3-4-year-olds and 5-to-7-year-olds (see Table 1 for demographic variables). Groups were split at the age of 5 to ensure equal-enough sample sizes in order to maximize statistical power. This split also optimized the available data regarding the questionnaire data (e.g., 3-4-year-olds filled out a different version of the BRIEF compared to the 5-to-7-year-olds). The two age-groups were similar with respect to distribution of karyotype (χ^2 (2,72) = 2.088, p = .352) and recruitment strategy (χ^2

(2,72) = .185, p = .912). Differences between research groups (SCT vs controls) were investigated within the two age-groups in terms of age, gender, and parental education level. Within the 3-4-year-old group, the SCT group included significantly more boys but was similar to the control group with respect to age and parental education level. In the 5-to-7-year-old group, children with SCT were significantly older than controls but groups were similar in terms of gender and parental education level.

Ethics and Procedure

This study was approved by the Ethical Committee of the Leiden University Medical Center, Leiden, the Netherlands, and the Colorado Multiple Institutional Review Board in the USA. A team of researchers, consisting of child psychologists, research associates, and graduate students, were trained and supervised by professionals in the field of child psychology, certified and specialized in neuropsychological assessment. All primary caregivers signed a written informed consent prior to assessment. Children were tested either in a quiet room at the University (SCT: 53%, controls: 43%) or at home (SCT: 47%, controls: 57%) using written protocols detailing all procedures and verbal instructions to standardize assessments. Researchers from Leiden University were responsible for project and data-management (i.e., training and supervision of researchers, processing, and scoring of data). The primary caregiver (92% female) of the child completed the questionnaire in either Dutch or English using the online survey software Qualtrics (http://www.qualtrics.com/).

Table 1

Demographic Background of SCT Group and Control Group

	All ages			**3-4-year-olds**			**5-to-7-year-olds**		
	SCT	**Controls**	***p***	**SCT**	**Controls**	***p***	**SCT**	**Controls**	***p***
	n = 72	*n* = 70		*n* = 41	*n* = 44		*n* = 31	*n* = 26	
Age in years – *M* (*SD*)	4.83 (1.29)	4.52 (.99)	.120	3.85 (.61)	3.91 (.58)	.935	6.12 (.75)	5.58 (.37)	**.005**
Gender	M = 45	M = 31	**.030**	M = 28	M = 20	**.034**	M = 17	M = 11	.346
	F = 27	F= 39		F = 13	F = 24		F = 14	F = 15	
Parental education level[a] - median (range)	6 (4 - 7)	6 (2 - 7)	.586	6.5 (4-7)	6 (2-7)	.461	6 (4-7)	6 (2-7)	.965
Karyotype		N/A			N/A			N/A	
XXX	27			13			14		
XXY	30			20			10		
XYY	15			8			7		
Recruitment strategy (*n*)		N/A			N/A			N/A	
Information-seeking parents	31			17			14		
Prospective follow-up	23			13			10		
Clinically referred	18			11			7		
Recruitment site (*n*)									
The Netherlands	39	70		15	44		24	26	
Denver, USA	33			26			7		

Note. SCT: sex chromosome trisomies; M = male, F = female; [a] = data from 2 primary caregivers (1 SCT, 1 control) was missing due to non-completion of questionnaires.

Instruments

Executive Function Skills

Global executive function skills were measured with the Minnesota Executive Function Scale (MEFS App™): a standardized performance-based assessment of global executive function skills, designed for children ages 2 and up, that is administrated on a touch-screen tablet (S. M. Carlson & Zelazo, 2014). Administration time is usually 2 to 6 minutes (average of 4 minutes). The reliability and validity are high and the app has been used in general and clinical populations (S. M. Carlson & Zelazo, 2014). The MEFS App™ is a comprehensive executive function measure that goes down to 2 years of age and spans throughout adulthood and provides a single graded scale based on the combined assessment of working memory, inhibitory control, and cognitive flexibility. The MEFS assessment has increasing difficulty and is sensitive to age and performance, according to an adaptive testing protocol based on the responses of the child. Children are asked to sort cards into two boxes according to one rule and then switch to sorting the same cards again using an opposite or conflicting rule (see Figure 1). It requires children to switch between rules and inhibit one's automatic response. Furthermore, working memory is required to remember the current rule(s) for each trail. Because of its adaptive testing protocol, the MEFS provides a sensitive assessment of each individual child and his/her global executive function skills. After finishing the task, a total score (0-100) is calculated based on an algorithm that takes both accuracy and response time into account, with higher scores reflecting better executive function skills. In analyses, either raw or standardized scores were used (see section on statistical analyses). Standardized scores were calculated differently depending on the recruitment site. For children from the Netherlands, scores from the current control group were used to calculate standardized scores (percentile scores). For children from the USA, scores from the general population were provided by the MEFS-app and converted into standardized scores (percentile scores).

Figure 1

Examples of Levels of the Executive Function Task

MEFS App™ Level 1 MEFS App™ Level 2 MEFS App™ Level 3

Note. First three levels of the MEFS App™. Children are instructed to sort cards into boxes based on a specific rule that increases in difficulty when a child progresses through the levels (displayed here: left: horses vs ducks, middle: large vs small, right: red vs. blue). Pictures from '*Minnesota Executive Function Scale App™ and Admin Portal User Guide*' (p.4) by S.M. Carlson and P.D. Zelazo, 2019, Reflection Sciences, Inc.™, St. Paul, MN. Reprinted with permission.

Verbal Executive Function Skills

To assess verbal executive function skills a measure of verbal fluency was used. Verbal fluency is commonly described as a measure of executive function in the context of verbal information (Pennington & Ozonoff, 1996) because it requires goal-directed behaviors such as cognitive flexibility, strategic planning, and error-monitoring (Diamond, 2013). For this study, the subtest 'Word Generation' of the NEPSY-II Developmental Neuropsychological Assessment was used (Korkman et al., 2007, 2010)). In this subtest children are asked to generate words within two specific categories ('animals' and 'food/beverages') as many as possible within a 60-second period for each category. Administrated answers were afterwards coded to yield either 0 points for an incorrect answer or 1 point for a correct unique answer. Higher scores represent higher levels of verbal fluency. Either summed raw scores or scaled scores were used in analyses (see section on statistical analyses). Scaled scores were derived from the manual, using the appropriate norm group depending on the language spoken by the child (Dutch or English).

Executive Functions in Daily Behaviors

The Behavior Rating Inventory of Executive Function (BRIEF) was used as an assessment tool of everyday executive functions (Gioia et al., 2000, 2003). It is developed to capture real-world manifestations of executive dysfunction, by focusing on children's everyday behaviors at home (Gioia et al., 2000, 2003) For the current study, primary caregivers completed either the BRIEF-P for a child aged 3;0 to 4;11 years (*n* = 84) or the BRIEF school-age for a child aged between 5;0 and 6;11 years (*n* = 54). A small subset of children (aged 5;0-5;11) fell within the appropriate age-range of both questionnaires. For future follow-up purposes caregivers of these children completed the BRIEF school-age. Both questionnaires in the Dutch and US version have satisfactory internal consistency (ranging from .78 to .98), test-retest reliability (ranging from .72 to .90) and convergent and discriminant validity (for the exact values see the appropriate manuals; US: Gioia et al., 2000, 2003; NL: Huizinga & Smidts, 2010; Van der Heijden, Suurland, de Sonneville, & Swaab, 2013). The BRIEF-P and BRIEF school-age comprise 63-item and 86-item rating scales respectively, with a 3-point rating including never, sometimes, and often. Both questionnaires have a total score (Global Executive Composite: GEC) with two or three indices, subdivided into multiple subscales. The overlapping subscales present in both BRIEF questionnaire versions include: Inhibit, Shift, Emotional Control, Working Memory, and Planning and Organization. Additional subscales for the BRIEF school age were Monitor, Organization of Materials, and Initiate. To compare all children across ages independently of which BRIEF version was administrated, for each child total scores were divided by the specific number of items to create mean scores. Higher scores indicate more difficulties. Either summed raw scores or standardized scores (T-scores) were used in analyses (see section on statistical analyses). Standardized scores were derived from the BRIEF(-P) manual, using the appropriate norm group depending on the recruitment site (The Netherlands or USA).

Intellectual Functioning

To control for potential group differences due to overall differences in intelligence, full scale IQ was estimated with a shortened version of the Wechsler Preschool and Primary Scale of

Intelligence – third edition (English version: WPPSI-III (Wechsler, 2002); Dutch version: WPPSI-III-NL (Wechsler, 2010)). This short version with four subtests (Vocabulary, Similarities, Block Design, and Matrix Reasoning) has been found to provide a valid and good estimation of the full scale intelligent quotient (Hurks et al., 2015). Standard IQ-scores were derived from manual, using the appropriate norm group depending on the recruitment site (The Netherlands or USA).

Statistical Analyses

Some data were missing due to outliers or technical dysfunction. Resulting from this, sample sizes varied from 60 to 72 SCT children and 67 to 69 controls per analysis. Data were analysed using IBM SPSS version 25. Demographic characteristics were compared with independent sample *t*-tests and Chi-square tests. As preliminary analysis, to examine whether recruitment site was relevant to executive function outcomes, *t*-tests were used to examine mean group differences within the SCT group (Dutch vs US) and mean group differences within the control group (Dutch or US-referenced norms).

For each executive function measure, the following analyses steps were taken. First, to test the hypothesis that executive functions were dependent on age, a linear approach using correlation analyses was used to maximize statistical power. Because raw scores were used in these analyses, they needed to be corrected for recruitment site. We used PROCESS, a bootstrapping, nonparametric resampling procedure (for further information see Hayes, 2009, 2017), to control for the potential role of recruitment site in the SCT group. Subsequently, if significant effects of age on EF measures were found, subsequent ANOVAs per age-group were performed to identify group differences at specific ages using standardized norms of the EF measures. These ANOVAs were carried out as post-hoc tests to analyze the differences between SCT and control within the different age-groups (3-4-year-olds and 5-to-7-year-olds) and thus only included those variables that were found significantly related to age. Thirdly, a correlation analysis with the standardized executive function measures (MEFS, NEPSY, BRIEF) and FSIQ was performed to examine the influence of IQ on

the results. If significance was revealed, post-hoc analyses were performed using ANOVAs to determine the specific role of IQ in executive functions.

Finally, two MANOVA's were performed to compare executive function measure outcomes (dependent variables, standardized scores) for the influence of recruitment strategy and karyotype, as a marker of data quality and representivity for the entire SCT group.

For correlation analyses with age, Pearson's product moment correlation coefficient was used. Level of significance was set at $p = .05$. For all significant effects, Cohen's *d* addressed effect size (.2 = small effect; .5 = medium effect; .8 = strong effect, Cohen, 1977).

Results

Preliminary Analyses

Preliminary analyses revealed minimal evidence of site effects on executive function scores (see Appendix A for the exact results). Within the SCT group, Dutch and American children did not differ on executive functions. Further, children in the control group did not differ from normative Dutch and US scores, with the exception of verbal executive function scores on the NEPSY subtask. Taken together these results provide the support to pool SCT children together and treat them as a singular group of SCT children and that controls are representative controls for both clinical samples.

Role of age in executive functions

The results from the correlation analysis between the variable 'age' and executive function parameters are shown in Table 2. Recruitment site was included as a covariate in the analyses but there were no significant interaction effects (see Appendix B and Table B1 for the exact results), thus indicating that recruitment site (The Netherlands or US) did not influence the results.

Within the SCT group, there were significant correlations between age and global executive function skills, verbal executive function skills, and different aspects of executive functions in daily life including emotional control and working memory. Most, but not all, correlations were also significant in the control group. For global executive function skills, working memory, and plan and organizing, the strengths of correlations differed significantly between research groups (see Table 2).

For the other domains, the strength of correlations did not differ significantly between the SCT and control group. These results indicate that age is an important factor in executive function (problems), with differential presentation across ages, as is visible in Figure 2.

Table 2

Correlations Between Age and Executive Functions for the SCT and Control Group

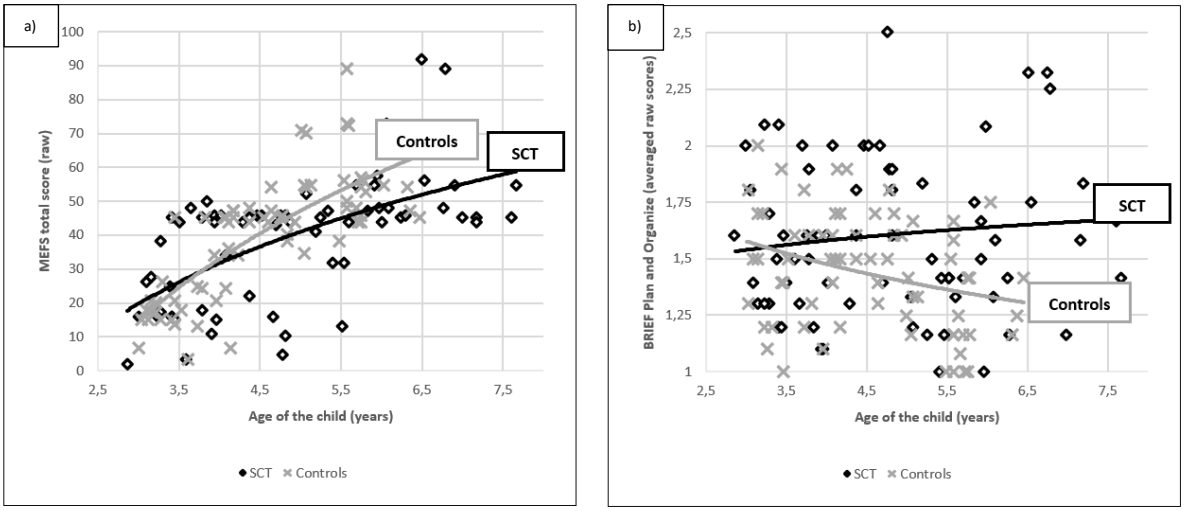
	SCT *age* *r*	CG *age* *r*	Fisher *r* to *z* transformation *z*	*p*
1. Global executive function skills (MEFS)	.590***	.764***	-1.810	**.035**
2. Verbal executive function skills (NEPSY)	.728***	.702***	.294	.384
3. Overall executive functioning (BRIEF GEC)	.418**	.265*	.998	.159
4. Inhibit (BRIEF)	.195	.104	.535	.296
5. Shift (BRIEF)	.294	.100	1.164	.122
6. Emotional control (BRIEF)	.455**	.312**	.966	.167
7. Working memory (BRIEF)	.422**	.154	1.694	**.045**
8. Plan and organize (BRIEF)	.128	-.325**	2.677	**.004**

Note: Higher scores reflect better performance for MEFS and NEPSY assessments, lower scores on the BRIEF reflect better functioning. Raw scores were used in analyses; in the SCT group, recruitment site was included as covariate. Abbreviations: SCT, sex chromosome trisomy; CG, control group; MEFS, Minnesota Executive Function Scale; NEPSY, NEPSY-II Developmental Neuropsychological Assessment; BRIEF, Behavior Rating Inventory of Executive Function; GEC, General Executive Composite.

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

Figure 2

Scatterplots of Child's Age Against Executive Function Scores for SCT and Control Group



Note. A) Global executive function skills (measured with MEFS) with higher scores reflecting better performance, B) daily life problems with planning and organizing (measured with BRIEF questionnaire) with lower scores reflecting better functioning. Separate lines represent correlation lines, displayed for each research group separately (SCT, controls).

Age-Specific Group Differences in Executive Functions

As age appeared to be associated with executive functions and showed differential patterns across ages and domains (for an illustration of these specific relations see Figure 2), specific age-groups (3-4 year-olds and 5-to-7-year-olds) were examined to identify impairments in executive functions at specific ages.

3-4-Year-Olds

There were significant group differences found for executive functions between 3-4-year-old children with SCT and controls (see Table 3). Children with SCT had significantly lower verbal executive function skills than the control group (medium effect size). For daily life behavior, parents of 3-4-year-old children with SCT reported, on average, more executive function difficulties in daily life compared to controls, also with great variability. A significant group difference was found for

working memory with medium effect size. Worth noting is that the domain of emotional control was trend significant (p = .067), with a medium effect size, as well as total general daily life difficulties (p = .066). With regards to global executive function skills assessed with a task, there was no significant group difference between the SCT and control group (p = .511).

5-to-7-Year-Olds

There were significant group differences for executive functions between 5-to-7-year-old children with SCT and controls (for results see Table 3). On the neurocognitive tasks, children with SCT had significantly lower global executive function skills and lower verbal executive function skills compared to controls, with large effect sizes. For executive function difficulties in daily life behavior, parents of 5-to-7-year-old children with SCT reported, on average, more executive function difficulties in daily life, compared to controls, with difficulties on almost all behavioral domains. Effect sizes range from *medium* (d = .6 for Initiate and Monitor) to *large* (d = 1.0 for Working Memory). Worth noting is the greater variance in scores in the SCT group on almost all BRIEF subdomains compared to controls.

Table 3

Age-Specific Differences Between Research Groups on Executive Function Domains

	3-4-year-olds					**5-to-7-year-olds**				
	SCT	**Controls**	**Statistical test results**			**SCT**	**Controls**	**Statistical test results**		
	M (SD)	*M (SD)*	*F*	*p*	*d*	*M (SD)*	*M (SD)*	*F*	*p*	*d*
Neurocognitive skills										
Global executive function skills (MEFS)	39.54 (24.40)	35.89 (25.31)	.436	.511	-	58.93 (19.47)	74.80 (14.84)	10.371	**.002**	.9
Verbal executive function skills (NEPSY)	10.59 (2.21)	11.86 (2.36)	5.555	**.021**	.5	8.71 (3.09)	11.31 (2.78)	10.449	**.002**	.9
Overall executive functions (BRIEF GEC)										
Total daily life executive functioning	55.02 (11.75)	50.77 (9.07)	4.608	.066	.4	54.29 (11.99)	44.35 (9.64)	11.592	**.001**	.9
Domains of executive functions (BRIEF indices)										
Inhibitory-self-control	52.66 (11.37)	49.81 (8.54)	1.691	.197	-	53.40 (14.17)	43.65 (10.65)	8.255	**.006**	.8
(Emergent) Metacognition	56.15 (11.34)	51.35 (9.49)	4.439	**.038**	.5	55.33 (13.02)	45.31 (9.14)	10.787	**.002**	.9
Flexibility	54.27 (12.45)	50.49 (9.44)	2.475	.120	-	N/A	N/A			
Behavioral specific executive functions (BRIEF subscales)										
Emotional control	53.56 (12.33)	49.30 (8.44)	3.441	.067	.4	56.55 (12.83)	44.85 (9.80)	14.512	**.001**	1.0
Working memory	56.95 (11.53)	51.09 (10.13)	6.134	**.015**	.5	56.38 (11.26)	46.27 (9.34)	13.304	**.001**	1.0
Plan and organize	54.10 (11.29)	51.63 (8.30)	1.313	.255	-	50.58 (10.59)	43.62 (6.68)	8.423	**.005**	.8

Initiate (only 5+)	N/A	N/A	53.10 (11.22)	47.15 (8.04)	4.928	**.031**	.6
Organization of materials (only 5+)	N/A	N/A	52.33 (12.83)	48.96 (9.51)	1.216	.275	-
Monitor (only 5+)	N/A	N/A	49.68 (10.35)	45.54 (8.26)	2.709	.105	-

Note. Higher scores reflect better performance for MEFS and NEPSY assessments, lower scores on the BRIEF reflect better functioning. Different standardized scores are used for each measure: MEFS included percentile scores; NEPSY included scaled scores with a mean of 10, SD of 3; BRIEF included T-scores with a mean of 50, SD of 10. Abbreviations: SCT, sex chromosome trisomy; CG, control group; MEFS, Minnesota Executive Function Scale; NEPSY, NEPSY-II Developmental Neuropsychological Assessment; BRIEF, Behavior Rating Inventory of Executive Function; GEC, General Executive Composite.

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

Role of IQ in Executive Functions

Results from the independent samples *t*-test showed that children with SCT had a significantly lower full-scale IQ (*M* = 95.51, *SD* = 19.75) than controls (*M* = 109.31, *SD* = 13.242), *t*(115)=-4.752, *p* < .001. Within the SCT group, no significant correlation was found between IQ and global executive function skills (*r* = -.084, *p* = .514). In contrast, IQ was significantly associated with verbal executive function skills (*r* = .497, *p* < .001) and daily life executive functions (BRIEF GEC score and all five BRIEF subscales; significant *r*-values ranging from -.299 to -.271).

To evaluate the relevance of these findings, we reran previous analyses within separate IQ-groups (children with SCT and below average IQ vs children with SCT and average IQ) as compared to controls (see Table 4 for the results). In both IQ groups, significant group differences were found between SCT and controls on almost all parameters, showing that difficulties with executive function were found across the range of intelligence levels and were not limited only to those children with below average IQ (also see Appendix C for additional analysis).

Role of Karyotype and Recruitment Strategy

In terms of the comparability of karyotypes, there were no significant group differences found between the three different karyotypes on executive functions (Pillai's trace = .160, *F*(6,104) = 1.507, *p* = .183). Which karyotype a child carried did not appear to affect the degree of executive functions, both in neurocognitive performance or in daily life behavior. Finally, to examine whether ascertainment method was relevant to the increased risk for executive function difficulties in children with SCT, a MANOVA was performed with executive functions (MEFS total score, NEPSY total score, BRIEF General Executive Composite score) as dependent variables and recruitment strategy within the SCT group (prospective follow-up, information seeking parents, clinically referred cases) as independent variable. There was no effect of ascertainment on executive functions (Pillai's trace = .190, *F*(3,104) = 1.822, *p* = .102): how children enrolled in the study did not appear to affect the degree of executive functions, both in neurocognitive assessment as in daily life behavior.

Table 4

Group Differences Between Controls and SCT Children Across IQ-Levels

	Controls	**SCT**		**Statistical test results**			
		IQ < 85	**IQ > 85**	**Controls vs SCT**		**Controls vs SCT**	
	N = 67	*N* = 17	*N* = 50	**IQ < 85**		**IQ > 85**	
	M (SD)	*M* (SD)	*M* (SD)	*F*	*d*	*F*	*d*
Verbal executive function skills (NEPSY)	11.71 (2.53)	7.46 (3.55)	10.39 (2.22)	27.239***	1.4	8.173***	.6
Overall executive functions							
General executive composite (GEC)	48.21 (9.84)	56.88 (10.68)	52.74 (11.80)	10.177***	.8	5.134**	.4
Behavioral specific executive functions							
Emotional control scale	47.36 (9.10)	55.94 (11.75)	53.66 (12.95)	10.676***	1.1	9.690***	.6
Working memory scale	49.27 (10.19)	57.94 (12.28)	55.26 (10.98)	9.021***	1.1	9.79***	.6
Plan and organize scale	48.84 (8.58)	54.65 (9.45)	51.24 (8.53)	5.974**	.9	1.948	-

Note. SCT: sex chromosome trisomy; CG: control group; below-average IQ: estimated full scale intelligence quotient below 85; average IQ: estimated full scale intelligence quotient above 85.

Level of significance: * $p < .1$, ** $p < .05$, *** $p < .01$.

Discussion

The present study investigated emerging executive functions in young children with SCT compared to population-based controls. We assessed whether children with SCT already show an increased risk for executive function difficulties at a young age. Core to the study was the inclusion

of a large international group of children with an extra X or Y chromosome between the ages of 3 to 7 years of which the majority had a prenatal diagnosis, which could provide insight in the developmental impact of SCT from a prospective point of view. Also critical to discussion of results is the acknowledgement of the variability in the SCT group and marked overlap with the control group such that many participants in the SCT group had scores that were similar or even improved compared to some individuals in the control group. However, statistical analyses of group differences are important as they help to delineate specific domains affected by SCT in order to understand how to support and to develop treatments for the proportion of individuals with SCT whose challenges in these areas are clinically significant such that they affect daily functioning and quality of life.

Our results revealed that children with SCT are at increased risk for problems with emerging executive functions, from as early as 3 years old, and that those problems appear more pronounced at an older age. Furthermore, impairments in executive functions appear broader than the language domain alone, extending to other areas as well, suggesting that impaired executive functions are part of the SCT neurodevelopmental profile, even when intelligence levels are in the typical range. To illustrate, specific difficulties are found for 3-to-4-year-old children with SCT in the area of verbal fluency and working memory. Children with SCT aged 5 to 7 years experienced more and broader executive function impairments than their peers, showing difficulties with global executive functions, verbal fluency, cognitive flexibility, emotional control, working memory, and planning and organizing. This is the first study showing that there is a developmental impact of SCT on emerging executive functions before the age of 7 years and that children with SCT are at significant risk for difficulties with executive functions in early childhood. Our findings add to the already existing literature done with older participants (Ross et al., 2008, 2009, van Rijn et al., 2008; Lee et al., 2011, Lee et al., 2015).

The increased risk for emerging executive function difficulties in children with SCT indicates that their ability to show purposeful, goal-directed, and problem-solving behavior is affected, from

as early as 3 years old. The impact for these children is significant, given that preschool executive functions are vital for school readiness (Blair & Razza, 2007), putting children with SCT at a substantial disadvantage at school entry. Furthermore, executive functions continue to be an important factor throughout childhood with regards to academic success, given that early executive functions also predict math and reading competence (Gathercole et al., 2004). Next to school readiness and academic success, adequate executive functions also impact psychological well-being, considering that impairments in executive functions has been linked to various symptoms of psychopathology in the general population, including both internalizing and externalizing behavioral problems (Kusche et al., 1993). Social, emotional, and behavioral problems are frequently reported in the SCT population (Samango-Sprouse et al., 2019; Tartaglia et al., 2020; Tartaglia et al., 2010, 2012) and our results suggest that emerging executive functions could be one of the key components in explaining the variability as well as the increased risk for psychopathology in this genetically at-risk group. Previous studies using older samples have already provided some evidence for this hypothesis showing a link between impairments in executive functions and social-emotional and behavioral problems (Skakkebæk et al., 2017), psychotic symptoms (van Rijn et al., 2009), and ADHD symptoms (Lee et al., 2011). Future studies should further investigate the relationship, both cross-sectionally as well as longitudinally, between emerging executive functions and psychological functioning in this young population of SCT.

Our study results also underline the importance of a developmental approach with regards to neurocognition in early childhood. Albeit we studied these children cross-sectionally, our results showed that increasing age is associated with more prominent and broader executive function difficulties in children with SCT. Deviations from controls were already evident from 3 years of age, but children in the 5-to-7-year-group showed more pronounced executive function difficulties (as illustrated by larger effect sizes) that appeared across multiple areas of functioning. Existing literature on the relation between age and executive functions in SCT is limited. However, our findings nicely complement one other study examining age-dependent effects of daily life executive

functions (Lee et al., 2015), in which children with Down syndrome and Klinefelter syndrome between the ages of 5 to 18 years old were compared to typically developing peers. The results from this study also showed more pronounced executive function difficulties with increasing age in the group children with an extra X chromosome, specifically in two areas of executive functions: plan/organize and initiate. Taken together, these findings suggest that the vulnerability for executive function difficulties in SCT might be already present from a young age but may not be limited to early childhood and is suggested to continue into later development. Looking from a neuropsychological perspective, we see a genetically at-risk group of children who show a differential pace in emerging neurocognitive functions, which could point to a suboptimal maturation of the brain and thereby possibly implicating future neurodevelopment. Albeit that our results show that a developmental approach provides additional insight into the impact of SCT, we acknowledge that our results were examined within a cross-sectional design and encourage the study of developmental trajectories of children with SCT using longitudinal studies to add validity to these results.

Within the age-specific executive function profiles, our finding on emotional control difficulties is worth highlighting. These results indicate that emotional control might be a relevant at-risk marker for young children with SCT, given that difficulties in this area are present at a young age (albeit trend significant at age 3) and appears to be one of the most pronounced weaknesses for children with SCT between the ages of 5 to 7 years. *Emotional control* represents an individual's ability to modulate emotional responses. Poor emotional control can be expressed as emotional lability or emotional explosiveness and caregivers usually describe these children as having overblown emotional reactions to seemingly minor events. From a developmental perspective, difficulties with adequately regulating and controlling your emotions have been linked to higher levels of social, emotional, and behavioral problems (Berkovits et al., 2017), highlighting the importance of emotion regulation abilities for quality of life. The findings of the current study complements existing literature who also described significant difficulties in emotional control and

regulation in adolescents and adults with SCT, expressed in behavioral problems (Lee et al., 2015) as well as physiological regulation difficulties (van Rijn, Barendse, et al., 2014). Moving forward, now that we have established that children with SCT are at increased risk for emotion regulation difficulties from an early age on, it is worthwhile to examine its developmental trajectory using a diverse set of measures, including those of the affective arousal system. These findings could provide further insight on the predictive value of emotion regulation difficulties in early childhood for later development, and also point to emotional control as a target for early treatment programs.

Important to note is the broad variation in executive functions observed between children with SCT in the current study. For clinical care, it is imperative to realize that having a specific genetic variation does not reliably predict what the exact outcome will be for any given individual. Thus, working in a clinical setting with children with SCT, professionals need to be aware of the variation in executive functions between children with SCT just as much as the developmental risk for impaired executive functions. From a young age, difficulties with (emerging) executive functions could be part of an individual's neurocognitive profile, even in the face of a typical intelligence, and therefore requires specific attention in assessment using age-appropriate and valid measures (including but not limited to neurocognitive tests, structured observations, development history interview). Identifying impairments in (specific areas of) executive functions can result in specific guidelines on what function needs to be supported during treatment. Given the significant relevance of executive functions on many developmental outcomes in childhood, specifically school readiness and achievement (Best et al., 2011), it is important to consider support options for preschool children with SCT who already experience difficulties in this area. Up to 48% of young children with SCT already receive early childhood intervention services before the age of 6, including preschool academic support, (Thompson et al., 2020) in which the area of executive functions could be addressed as well. Treatment and/or support could include training the specific executive function skill, using stronger-developed skills to compensate for the less-developed executive function, and/or adjusting the context to the limitations or the dysfunction itself by implementing tools or

lowering expectations. Empirical studies on executive function intervention in children with SCT are non-existent, but the study on effectiveness of executive function interventions in the general population is a promising but emerging field (Diamond & Lee, 2011). A recent meta-analytic review on the effectiveness of cognitive training in preschoolers (Scionti et al., 2020) showed that there is an overall effect of cognitive training in improving executive functions, especially in at-risk groups (ADHD or children with low socio-economic status), suggesting that those at risk might benefit more from stimulation than children without additional risks. However, Scionti and colleagues (2020) did not find an effect of cognitive training on additional outcomes, such as psychological or behavioral benefits. In sum, while these results indicate that executive function training might also be a valuable component in treating (emerging) executive function difficulties in children with SCT, it is crucial not to focus narrowly on improving executive functions alone, but also address the social, emotional, and behavioral development in addition to the social context in which a child with SCT grows up in (family and school).

While the results of the current study are promising and the size of the sample is noteworthy, especially since genetic population are difficult to recruit, the current study also has limitations that should be addressed. As mentioned previously, our results are based on a cross-sectional designed study. Longitudinal studies are crucial to add validity to our age-dependent results and could provide further insight in the developmental pathways of EF in children with SCT. Also, by collapsing across sex chromosomal trisomies we were not able to assess the specific contribution of karyotype (XXX, XXY, and XYY) on impairments in executive functions. Thirdly, the current study did not examine the effect of early testosterone hormone treatment on the neurocognitive profile in the SCT subgroup with XXY. Although treatment with testosterone might be considered to improve the physical implications of a micropenis, the evidence for potential benefits of early testosterone on (neuro)developmental outcomes in infants with Klinefelter syndrome is still limited (Aksglaede et al., 2020). We support the initiative of Aksglaede and colleagues (2020) who

call for a randomized and placebo-controlled trial with an adequately powered cohort sample: one of which is currently underway (PI Davis, NCT03325647).

Conclusion

In sum, the present study showed that when it comes to emerging (e.g., still-developing) executive functions, many (but not all) children with SCT experience reduced performance and everyday functioning , which seems to be present from a young age (3 years). There appears to be a broader and more significantly impaired executive function profile in older children with SCT, suggesting increasing impairments in executive functions with age. These impairments in executive functions are broader than the language domain alone, extending to other areas as well, including planning, emotional control, and working memory. The increased risk for impaired executive functions appears to be robust and present above and beyond differences in intelligence, karyotype, recruitment site, and recruitment strategy. This increased risk in early childhood might point to a suboptimal brain maturation in children with SCT. Additional research is warranted using a larger sample that also examines the predictive value of executive functions in terms psychopathology. Our data indicate that emotional control could be an important candidate. Clinically, the results from the study show that impairments in executive functions are part of the broad variation that can occur in SCT, even in the presence of typical levels of intelligence. It highlights the importance of early monitoring and screening of executive functions in preschool children with SCT, which may allow for preventive and early intervention to optimize developmental outcomes.

APPENDICES

Appendix A. Group differences on executive function parameters (standardized scores) in the SCT and control group.

Appendix B. Moderation effect of recruitment site in the SCT group.

Appendix C. Role of estimated IQ in the group differences between SCT and controls on executive function outcomes.

Appendix A. Group differences on executive function parameters (standardized scores) in the SCT and control group.

	SCT NL (N = 39)	**SCT US** (N = 33)		**Controls NL-ref group** (N = 69)	**Controls US-ref group** (N = 69)	
	M (SD)	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*
Neurocognitive skills						
Global executive function skills (MEFS)	52.56 (26.13)	41.94 (21.07)	.079	50.56 (29.36)	46.78 (18.78)	.373
Verbal executive function skills (NEPSY)	9.97 (3.19)	9.36 (2.16)	.409	11.65 (2.53)	12.51 (2.55)	**.049**
Overall executive functions problems (BRIEF GEC)						
Total daily life executive functioning	53.38 (11.24)	56.27 (12.36)	.303	48.35 (9.73)	48.79 (10.25)	.796
Behavioral specific executive functions (BRIEF subscales)						
Inhibit	49.69 (10.13)	51.88 (11.27)	.389	48.26 (8.83)	49.90 (8.12)	.258
Shift	53.62 (11.08)	55.18 (13.09)	.584	48.91 (10.23)	50.14 (10.43)	.486
Emotional control	54.00 (11.60)	55.85 (13.69)	.537	47.62 (9.16)	48.20 (9.84)	.721
Working memory	54.56 (10.96)	59.24 (11.41)	.081	49.27 (10.05)	51.64 (10.32)	.174
Plan and organize	52.36 (10.48)	52.85 (11.06)	.853	48.61 (8.61)	48.81 (7.53)	.885

Note. Different standardized scores are used for each measure: MEFS included percentile scores; NEPSY included scaled scores with a mean of 10, SD of 3; BRIEF included T-scores with a mean of 50, SD of 10. Abbreviations: SCT, sex chromosome trisomy; CG, control group; MEFS, Minnesota Executive Function Scale; NEPSY, NEPSY-II Developmental Neuropsychological Assessment; BRIEF, Behavior Rating Inventory of Executive Function; GEC, General Executive Composite.

Appendix B

Moderation Effect of Recruitment Site in the SCT Group

Analysis description. Bias-corrected bootstrapping analyses (PROCESS) were conducted to test for a moderating effect of the recruitment site (Dutch or US) on the relations between age and executive function parameters in the SCT group. There were no significant interaction (e.g., moderation) effect of recruitment site, revealing that the relation between age and executive function outcomes did not differ across sites. See Table B1 for the exact results.

Table B1

PROCESS Results on the Moderation Effect of Recruitment Site in the SCT Group in the Relation Between Age and Executive Function Outcomes

	Recruitment site (interaction effect)			
	b	*SE*	*t*	*p*
Global executive function skills (MEFS)	1.63	1.62	1.00	.320
Verbal executive function skills (NEPSY)	-.71	.64	-1.10	.275
Overall executive functioning (BRIEF GEC)	.03	.04	.89	.379
Inhibit (BRIEF)	.02	.05	.34	.735
Shift (BRIEF)	.02	.05	.46	.650
Emotional control (BRIEF)	.04	.06	.76	.451
Working memory (BRIEF)	.05	.05	1.05	.296
Plan and organize (BRIEF)	-.01	.04	-.03	.976

Note. Raw scores on all outcome parameters were used in the PROCESS analyses.

Appendix C

Role of Estimated IQ in Group Differences Between SCT and Controls on Executive Function Outcomes

To address the robustness of our results concerning the role of IQ, we also ran a MANOVA with the executive function parameters as dependent variables, research group as independent variable and included IQ as covariate. These results again showed a multivariate effect of research group (p = .004), next to a multivariate effect of IQ (p < .001). Thus, caregivers of children with SCT reported significant more daily life executive functions problems (while controlling for IQ), specifically emotional control (p = .017) and working memory (p = .022). Also, children from the SCT group performed significantly less well than controls on verbal executive functions (p = .017). Group differences were non-significant for inhibit (p = .969), shift (p = .199), and plan/organize (p = .331).

CHAPTER 4

Emotional reactivity and expressivity in young children with sex chromosome trisomies: Evidence from psychophysiological and observational data

Published as:

Kuiper, K. C., Swaab, H., Tartaglia, N., Cordeiro, L., & van Rijn, S. (2022). Emotional reactivity and expressivity in young children with sex chromosome trisomies: evidence from psychophysiological and observational data*, Child Neuropsychology*, 1-19, DOI: 10.1080/09297049.2022.2102161

Abstract

Although sex chromosomal trisomies (SCT) in children are highly prevalent and associated with an increased risk for neurodevelopmental difficulties including socio-emotional problems, little is known about underlying mechanisms that could drive this risk. Studying emotional reactivity and expressivity of young children with SCT in early childhood could identify deviations in early emotional development and potentially serve as risk markers to guide clinical care in developing interventions. Participants in the current study were 90 SCT children and 97 population-based controls, aged 1 to 7 years, who experienced a stress-inducing event in which physiological (heart rate) and observational data (expression of negative emotions) were collected. Results showed early disturbances in the emotion system of young children with SCT, in terms of blunted but prolonged emotional reactivity and a reduced emotional expressivity in response to stress. Further, the concordance between emotional reactivity (arousal response) and expressivity was significantly lower in SCT, compared to controls. Given the significant impact of emotions on adaptive day-to-day functioning, deviations in processing emotions could be an important underlying mechanism in explaining the heterogeneity and variability in developmental outcomes often described in individuals with SCT.

Emotional Reactivity and Expressivity in Young Children with Sex Chromosome Trisomies: Evidence from Psychophysiological and Observational Data

Sex chromosomal trisomies (SCT) are among the most common chromosomal duplications, with a high prevalence of 1-2 in 1000 births (Berglund et al., 2019; Bojesen et al., 2003; Groth et al., 2013; J. K. Morris et al., 2008). The chromosomal karyotypes include 47,XXY (Klinefelter syndrome) and 47,XYY (XYY syndrome) in males and 47,XXX (Trisomy X syndrome) in females. Studying genetic conditions as SCT from pregnancy on provides an unique opportunity to prospectively examine potentially at-risk development and identify early mechanisms that can contribute to outcomes (later) in life. This is especially relevant in the SCT population, since these individuals have an increased risk for various (neuro)developmental difficulties, including behavioral, learning, and socio-emotional problems (for a review see Urbanus, van Rijn, & Swaab, 2020). To date, what drives these increased risks remains fairly unknown. To explain these neurodevelopmental difficulties, research has primarily focused on the area of information processing skills and has already identified difficulties in general intellectual functioning (albeit at the lower end of the typical range), social cognition, executive functions, and language (for a review on this topic, see van Rijn, 2019). Of equal importance is the perception and understanding of emotions, given that emotions are also crucial for our day-to-day functioning. Studying emotions in SCT is relevant: numerous studies have shown that individuals with SCT are vulnerable in their emotional development and often show difficulties in this area, including emotional outbursts (Visootsak & Graham, 2009), affective problems (Urbanus, Swaab, et al., 2020), and depressive symptoms (Tartaglia et al., 2010). Also, increased symptoms of psychiatric disorders associated with emotional difficulties such as autism spectrum disorders (ASD) are not uncommon in the SCT population (Ross et al., 2012; van Rijn, Stockmann, et al., 2014). Since most of previous studies have examined older children and adults and mostly with a 47,XXY karyotype, information about the early emotional development in young children (before the age of 8 years) across all three karyotypes is limited. However, it is needed to identify early risk markers that help explain the increased risk for psychopathology and potentially guide clinical care in

developing early and preventive interventions to support the overall development of these children. The current study aims to provide in this need for knowledge.

Important in the study of emotions, is that they are considered to be multifaceted and include whole-body processes, such as physiological and behavioral responses (Gross, 2013). Emotions involve person-situation interactions that compel attention and give rise to coordinated yet flexible responses that in turn modify the ongoing interaction (Gross, 2013). Thus, emotions serve a signaling function, in which they highlight events as relevant or irrelevant to an individual and help to identify which situations are attention-compelling (and which are not). To quantify the signaling function of emotions, *emotional reactivity* (also called affective arousal) can be assessed, which is the initial arousal response on a physiological level (Gross, 2013). After situations are signaled as relevant, self-regulatory processes activate the autonomic nervous system (Sapolsky, 2004), including the sympathetic nervous system (SNS) that stimulates increased respiratory rate and heart rate, preparing the body both physiologically and behaviorally to act (Porges & Furman, 2011). To illustrate: when in danger, an individual needs to attend their attention to the dangerous situation quickly (i.e., signal the event as relevant), which in turn will activate physiological changes, including heart rate acceleration, that enables a fight or flight response. The link with functional psychological outcomes in childhood day-to-day life is evident: sufficient emotional reactivity (in terms of SNS activity) in challenging situations is related to greater self-soothing, more attentional control, and greater capacity for social engagement (Blair & Peters, 2003; Calkins et al., 2002; Calkins & Keane, 2004). On the other hand, inadequate emotional reactivity has been linked to both childhood externalizing and internalizing behavior problems (T. Beauchaine, 2001; Boyce et al., 2001). In the SCT population, studies on emotions have primarily included behavioral measures and questionnaire data and only two other studies so far have examined direct psychophysiological indices of emotional reactivity and also yielded discordant results: the first that showed increased affective arousal in response to viewing emotion-evoking visual images (van Rijn, Barendse, et al., 2014) and the second that found a blunted affective arousal response to evoking social stimuli in young children with SCT

(Urbanus et al., n.d.); thus highlighting the importance of further investigation of emotional processes in SCT.

Emotional reactivity, following emotion perception and appraisal, serves a key role in psychosocial functioning. By signaling the demands of the environment, emotional reactivity enables the coordination of behavioral responses that in turn facilitate adaptive behavior (Gross, 2013). As a behavioral response, the *expression of emotions* serves an important social and communicative function (Greenberg, 2004), in that the display of (facial) emotions can elicit behavior in others which in turn influences the ongoing interaction. For example, showing fear can elicit others to approach for help, whereas showing anger can signal others to avoid and withdraw (Marsh et al., 2005). In young children, the frequency and intensity of emotional expressivity has been linked to the quality of social relationships (Diaz et al., 2017; Eisenberg et al., 1993) and the child's feelings of social competence (Waiden & Field, 1990). Individual differences in the expression of negative emotions were also found to be related to externalizing problem behavior in typical developing children (Eisenberg, Cumberland, et al., 2001), highlighting that the amount and intensity of emotion expressivity can have differential effects on person-situation interactions. So far, studies on emotional expressivity in individuals with SCT typically examined the behavioral consequences of inappropriate emotional expression, such as emotional outbursts (van Rijn & Swaab, 2020; Visootsak & Graham Jr, 2009), instead of examining the expression of emotions as the main focus of study. Even if studies examined emotional expressivity, it was usually done using self-reported data. The current study provides one of the first observational studies of emotional expressivity in children with SCT.

For adequate psychosocial functioning, a concordant system of matching emotional internal and external processes is key. When the overt display of emotions matches the internal arousal response (e.g., *emotional concordance*), it informs the environment on the internal state of the child which enables others to adequately responds to a child's needs (Robinson et al., 1997). Discordance however can significantly hinder the engagement of the environment and confuse others about

actual internal states (Mauss et al., 2011). In fact, caregivers and other social partners decide whether to engage with the child or to retreat from interaction following a child's display of emotion and behavior (Denham, 1998). Thus, the expression of emotions and the concordance with the physiological arousal response is important in terms of adaptive social and communicative functioning. Concordance between these two emotional constructs has not yet been studied in individuals with SCT.

To the best of our knowledge, this study is the first to examine these components of emotional development in young individuals with SCT. Studying children at this young age (before the age of 7) can provide insight in early "at-risk" development. It is not surprising that a well-attuned emotion system and emotional management skills are key milestones for social and cognitive functioning, with foundations in the earliest years of life (Gross, 2013). We propose that differences in the emotional development, i.e., being over- or underaroused or having a discordant display of emotions, might already emerge during early childhood in children with SCT, potentially laying the groundwork for other developmental difficulties. In sum, the current study aims to investigate early emotional development of young children with an extra X or Y chromosome, in terms of emotional reactivity and emotional expression and its concordance, using a standardized behavioral assessment of a stressful event. Key to this study is that use of direct, objective, and sensitive measures of the emotion system in terms of physiological (heart rate) and observational data in a large sample of young and predominantly prenatally diagnosed children with an extra X or Y chromosome. As many earlier studies with SCT included postnatally diagnosed participants, this study includes a wider range of phenotypic characteristics. We examine these constructs of the emotion system during a stress-inducing event, as opposed to a resting state, because it is precisely those situations that are potentially 'threatening' that elicit the emotional system to enable quick and adaptive responses.

Methods

Participants

The current study is part of a larger international study (the TRIXY Early Childhood Study, centred at Leiden University in the Netherlands, including research sites in the Netherlands and Colorado, in the United States of America [USA]). The TRIXY Early Childhood Study investigates the social, emotional, and behavioral development of young children with a trisomy of the X/Y chromosomes (TRIXY). For the current study, children aged 1 year to and including 7 years (at baseline) were included. Children with SCT were recruited from two sites: The Centre of Expertise for Trisomy of the X and Y chromosomes (TRIXY) in the Netherlands that recruited children from the Dutch-speaking countries in Western Europe (*n* = 42) and the eXtraordinarY Kids Clinic in Developmental Pediatrics at the Children's Hospital Colorado (CHCO) that recruited children from across the United States of America (USA, *n* = 48). Primary caregivers of children with SCT were contacted with the help of clinical genetics departments, pediatricians, and national advocacy or support groups for (parents of) individuals with SCT with recruitment flyers and postings on the internet and social media. Recruitment strategies for the SCT group resulted in three inclusion trajectories (see Table 1): a) 'information seeking parents', b) 'active prospective follow-up', and c) 'clinically referred cases'. Children in the control group were recruited from day care centers, public institutions, and elementary schools from the western part of the Netherlands. Inclusion criteria for all participants were that parents and/or children were Dutch- or English-speaking and children had no previous head injuries, severely impaired hearing or vision, and/or colour-blindness.

In this study, 90 children with SCT and 97 age matched population-based controls participated (see Table 1). The SCT group consisted of 30 girls with 47,XXX, 45 boys with 47,XXY, and 15 boys with 47,XYY. As for the timing of SCT diagnosis, 60 children (66.7%) had a prenatal diagnosis (i.e., because of [routine] prenatal screening, abnormal ultrasound findings, or advanced maternal age) and 30 children (33.3%) had a postnatal diagnosis (i.e., because of developmental delay, physical and/or growth problems, or medical concerns). Genetic testing results were reviewed to confirm sex

chromosome trisomy in at least 80% of cells. Children from the control group were not subjected to genetic screening, due to ethical considerations of blood testing. They were considered to be a representative sample of the general population. In addition, given the prevalence of SCT is ~1 in 1000, we reviewed the possible risk of having a child with undiagnosed SCT in our control group minimal and acceptable.

Background Information of Participants

Global intellectual functioning (GIF) was assessed with the Bayley Scales of Infant and Toddler Development (N_{SCT} = 27, $N_{control}$ = 31, Bayley, 2006) in children aged 1 to 2 years, and the short-version of the Wechsler Preschool and Primary Scale of Intelligence third edition (N_{SCT} = 61; $N_{control}$ = 64; WPPSI; (Wechsler, 2002)) in children aged 3 years or older. GIF scores for 4 children in the SCT group were missing. There was a significant difference in average full-scale intelligence scores between the SCT and control group, $t(181) = -4.009$, $p < .001$, $d = .6$. Although both groups on average scored within the average range, the SCT group scored lower (M = 96.63, SD = 18.14) than the control group (M = 106.27, SD = 14.32). Secondly, parental education level was assessed according to the Hollingshead criteria and ranged from category 1 (no formal education) to 7 (graduate professional training) (Hollingshead, 1975). When the child was raised by two parents (95%), educational level was averaged over both parents. Median parental education level was the same (6) in the SCT and control group (p = .637).

Ethics and Procedure of the Assessment

This study was approved by the Ethical Committee of the Leiden University Medical Center, Leiden, the Netherlands, and the Colorado Multiple Institutional Review Board (COMIRB) in the USA. Researchers from Leiden University were responsible for project and data-management (i.e., training and supervision of researchers, processing and scoring of data). A written informed consent was signed by the primary caregivers of all participating children. Before the lab or home visit, participants were explicitly prepared with a visual information brochure and a copy set of the electrodes for the physiological assessment. Research took place in a quiet, stimuli-low room either

Table 1

Demographic Characteristics of the SCT and Control Group.

	SCT *n* = 90	Controls *n* = 97	Group differences
Age in years – *M (SD*)	3.74 (.20)	3.62 (.17)	$t(185) = .496$, $p = .621$
Gender	M = 60, F = 30	M = 43, F = 54	$X^2 (1) = 9.414$, $p < .01$
Global intellectual functioning – *M (SD)*	96.63 (18.14)	106.27 (14.32)	$t(181) = -4.009$, $p < 0.001$
Range	59 - 138	72 – 140	
Parental education level – median (range)	6 (3.5 - 7)	6 (2 to 7)	$p = .637$
Recruitment strategy - *n* (%)			
Information-seeking parents	43 (47.8%)		
Prospective follow-up	28 (31.1%)		
Clinically referred	19 (21.1%)		

Note. For children aged 1;0 to 2;0, the Cognitive Composite score from the Bayley-3 (Bayley, 2006) was used as global intellectual functioning; for children aged 3;0 to 7;11, the estimated Full Scale IQ-score from the short version of the WPPSI-III (Wechsler, 2002) was used as global intellectual functioning. Both scores have a mean of 100 and standard deviation of 15.

at the university or in the family home, using written protocols detailing all procedures and verbal instructions to standardize assessments. Children were given time to familiarize before and after the electrodes were applied by playing an age-appropriate game, while seated in a car set to have a stable and framed position suited for physiological measurement.

Measures

Physiological Arousal

Two electrodes were attached at the top center of the chest (10 centimeters below the suprasternal notch) and the bottom left of the ribs (10 centimeters above the bottom of the rib

cage). Heart rate was recorded continuously during baseline and the unpredictable toy paradigm with AcqKnowledge (version 5.0.2, BIOPAC Systems Inc.). Recording was acquired through an Electrocardiogram amplifier (ECG100C) and a BIOPAC data acquisition system (MP150 Windows) with a sampling rate of 1.000 Hz. In AcqKnowledge a 0.5 Hz highpass filter and 50 Hz notch filter were applied to stabilize the ECG signal. Recorded physiological data was further processed by inspecting the detected R peaks in PhysioData Toolbox version 0.5.0 (Sjak-Shie, 2020). Motion artifacts were visually identified and excluded from the data. Heart rate data (beats per minute: BPM) were summarized in 30-second epochs in concordance with the behavioral data.

Baseline

To measure baseline, children watched a 3 minute video of a fish tank, which has been shown to be an adequate measure of resting state (Piferi et al., 2000). Heart rate (in BPM) over the course of the video was analyzed in epochs of 30 seconds each and the epoch in which children had the lowest heart rate was identified as representing resting state. This was done on group level, for the control group and the SCT group separately.

Emotional Distress during Stressful Event

The standardized Unpredictable Mechanical Toy Task from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith et al., 1999) was chosen as a stressful event, to induce general emotional distress. It contains elements of non-social novelty and intrusiveness in which the toy (i.e., stressor) is, given the context, relatively inescapable. For the current study, a remote-controlled robot was chosen as the distressing stimulus (also see Appendix A), which has also been used in other studies with clinical groups examining emotional processing (e.g. in ASD: Zantinge et al. (2018), in mood disorders: Savory et al. (2020)). The procedure of the task was executed following the Lab-TAB manual. Caregivers were instructed to sit in the room out of direct sight, filling out questionnaires or reading magazines, and to remain as uninvolved as possible while displaying a neutral face. An experimenter entered the room in a white laboratory coat and protection glasses placing a (novel to the child) robot 1.5 meters away from the child. The robot made three

approaches of 30 seconds each, starting by walking towards the child, stopping 15 centimeters in front of the child moving its arms and emitting noise. Then, the robot walked backward, pausing for 5 seconds before moving forward again, repeating this sequence three times in total (i.e., stress phase). During the entire task, the experimenter did not make any eye-contact or communicate with the child. After stress phase, the experimenter left the room with the robot and the caregiver was instructed to sit with the child and watch an animated video of 2 minutes together (i.e., recovery phase). During recovery, caregivers were allowed to sooth and comfort their child if necessary, but the child stayed in the car seat for the remaining time of the video to allow for stability in the assessment of physiological recovery. The entire procedure was videotaped from two angles.

In case caregivers judged the experiment as too stressful for their child, they were allowed to stop the experiment and move forward to the recovery phase. Twenty-two percent of the parents (24 SCT, 18 controls) requested for an early termination. A total of 145 children completed the full experiment, of which 66 were children with SCT and 79 were controls. Non-completers were significantly younger (M = 2.92, SD = 1.52) than children who finished the experiment (M = 3.90, SD = 1.78), but there were no other significant group differences, e.g., in terms of research group (X^2 (1) = 1.763, p = .184) or gender (X^2 (1) = .160, p = .690).

To analyze the reactivity and recovery of the physiological system, four moments from the toy task were chosen for analysis: 1) initial stress (the first 30 seconds of the stress phase), 2) prolonged stress (the final 30 seconds of the stress phase), 3) initial recovery (the initial 30 seconds of the 120-second recovery phase), and 4) extended recovery (the 30-second period between 60-90 seconds of the 120-second recovery phase).

Observational Coding of Emotional Expression

Videos of the unpredictable mechanical toy task were subsequently coded in 10-s epochs (with sound on) for emotional expression following the coding instructions from the Lab-TAB manual (Goldsmith et al., 1999) and the facial and bodily indicators of three basic emotions (fear, sadness, and anger) as described in the Facial Action Coding System (FACS; Ekman & Friesen (1976)). The peak

intensity of the emotions was coded within each 10-s epoch to catch the burst of facial and bodily expression during these intervals. Facial and bodily indicators of the three emotions were scored on a 4-point scale (0–3): neutral (0 – no sign of facial or bodily emotion), mild (1 – one observable facial or bodily sign), moderate (2 – two observable signs) and severe (3 – more signs). The scores were averaged across the available epochs per participant. A composite negative emotionality score was calculated derived by summing these averages. Inter-rater reliability (IRR) was assessed using a two-way mixed, absolute agreement intra-class correlation model (Hallgren, 2012). The IRR was substantial for emotional expression (intra-class correlation coefficient (ICC) = 0.81, $p < 0.001$). Four trained independent coders scored the recorded videos of which 25% were double coded. IRR was monitored continuously in regular consensus meetings. Discrepancies were discussed within the team to obtain a final consensus score. Distress vocalizations, as described in the Lab-TAB manual, were not included in the analyses since our study focused on the visual observable expression of emotion.

Data Analysis

Due to missing data, the number of participants differed across analyses. Reasons for missing data included technological difficulties only, such as hardware or saving fail or a blocked camera view. For physiological data and the observational data taken together, data was missing from 12 children with SCT and 9 children with TD. Analyses were performed by excluding participants with missing values analysis-by-analysis. After inspection for outliers and normality checks regarding all data, no children were excluded.

First, baseline heart rate levels (BPM) between the controls and the SCT group were analyzed with an independent samples *t*-test, as well as within-group comparisons between the different karyotypes (ANOVA). Next, a GLM repeated measures analysis was performed with the between subject factor Group (SCT, controls) and the within-subjects factor Task (initial stress, prolonged stress, initial recovery, and extended recovery). Heart-rate data during the stressful event was corrected for baseline heart rate. To further analyze the heart rate pattern over time and potential

group differences, paired sample *t*-tests were done for each research group separately. Subsequently, emotional expression was examined with a GLM repeated measures analysis with the between subject factor Group (SCT, controls) and the within-subjects factor Task (initial stress, prolonged stress). Also, to examine the concordance between expression and arousal response (Pearson) correlation analyses were performed between the arousal response from initial to prolonged stress phase and total emotional expression separately for both groups. When aforementioned analyses revealed significant group differences, of secondary interest to the study was whether these group effects were dependent of age. The moderating effect of age was assessed using PROCESS, a bootstrapping, nonparametric resampling procedure (Hayes, 2012). Bootstrapping analysis with 5000 resamples was done to test for a significant moderating effect using the SPSS macro developed by Hayes (2012). Outcome variables and moderator variable (i.e. child's age) were centered. In this analysis, the moderation effect is significant if the 95% bias corrected confidence interval for the moderator effect does not include zero. Finally, additional MANOVA's were performed to examine the quality of the data and its representativeness based on a number of key background variables (karyotype, recruitment strategy, recruitment site). Level of significance was set at $p = .05$. For all significant effects, Cohen's *d* addressed effect size (.2 = small effect; .5 = medium effect; .8 = strong effect, Cohen, 1977).

Results

Psychophysiological Arousal During Baseline

Baseline heart rate levels did not differ between children with SCT ($M = 103.06$, $SD = 17.52$) and controls ($M = 100.94$, $SD = 13.90$) ($t(112) = .761$, $p = .448$). Also, there were no significant differences between the three different karyotypes in the SCT group ($F(2,76) = 2.702$, $p = .074$): the mean baseline heart rate was 96.83 ($SD = 16.50$) for boys with 47,XYY, 108.97 ($SD = 16.59$) for boys with 47,XXY, and 101.84 ($SD = 18.34$) for girls with 47,XXX.

Psychophysiological Arousal in Response to Stressful Event

GLM repeated measure analysis revealed a significant main effect of Task ($F(3,129) = 23.436$, $p < .001$, eta = .353), a significant effect of Group ($F(1,131) = 4.643$, $p < .05$, eta = .034), and a significant Task * Group interaction effect ($F(3,129) = 3.183$, $p < .05$, eta = .069). In other words, physiological arousal changed in response to a stressful event, but this pattern differed between children with SCT and controls (see Figure 1). Within group comparison revealed a differential reactivity and recovery pattern between children with SCT and controls (see Table 2). When faced with an unexpected stressor, children with SCT showed a weaker arousal response compared to controls (indicating a blunted response) and their recovery took longer compared to controls who showed an immediate initial recovery response.

Table 2

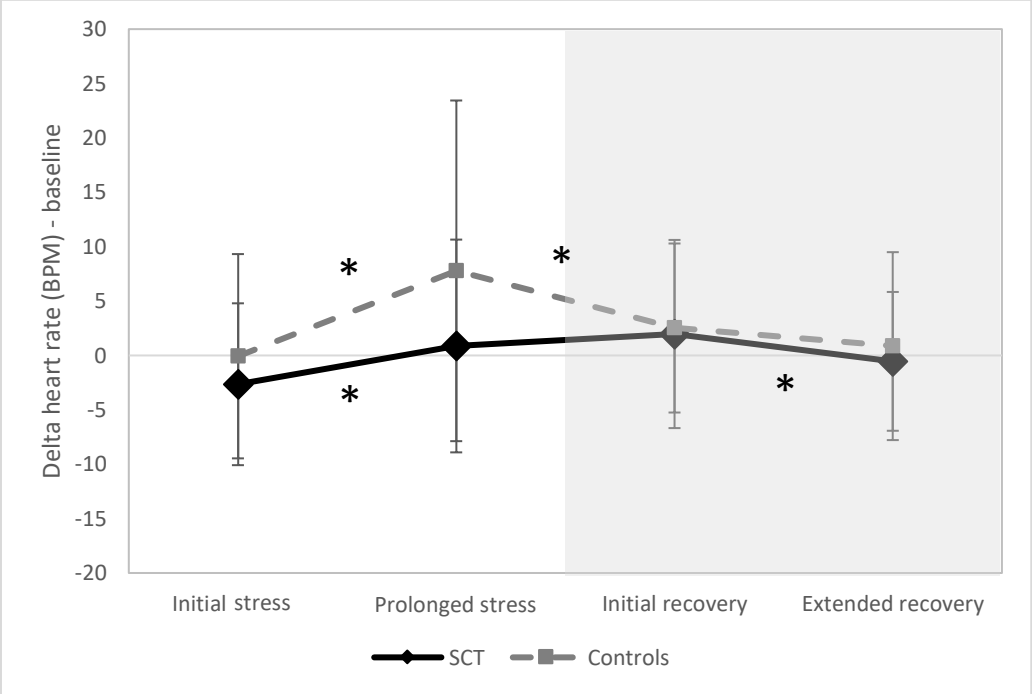
Within Group Increases in Arousal (Heart Rate) in Response to Stressful Event.

	SCT	**Controls**
Initial stress to prolonged stress	$t(60) = -3.996$, $p < .001$, $d = .5$	$t(72) = -5.966$, $p < .001$, $d = .7$
Prolonged stress to initial recovery	$t(60) = -1.064$, $p = .292$	$t(72) = 3.142$, $p < .01$, $d = .4$
Initial recovery to extended recovery	$t(60) = 2.686$, $p < .01$, $d = .4$	$t(72) = 1.939$, $p = .056$

Note. Effect sizes displayed in Cohen's *d*. Abbreviations: SCT: Sex Chromosomal Trisomies.

Figure 1

Physiological Reactivity and Recovery Patterns for Sex Chromosome Trisomies (SCT) group and Control Group during Stressful Event



Note. An asterisk between two arrows indicates a significant mean difference ($p < .05$) between two time points (for results see Table 2). The white area in the graph represents the toy task "stress" phase, the grey area represents the recovery phase.

Role of Age in Psychophysiological Arousal

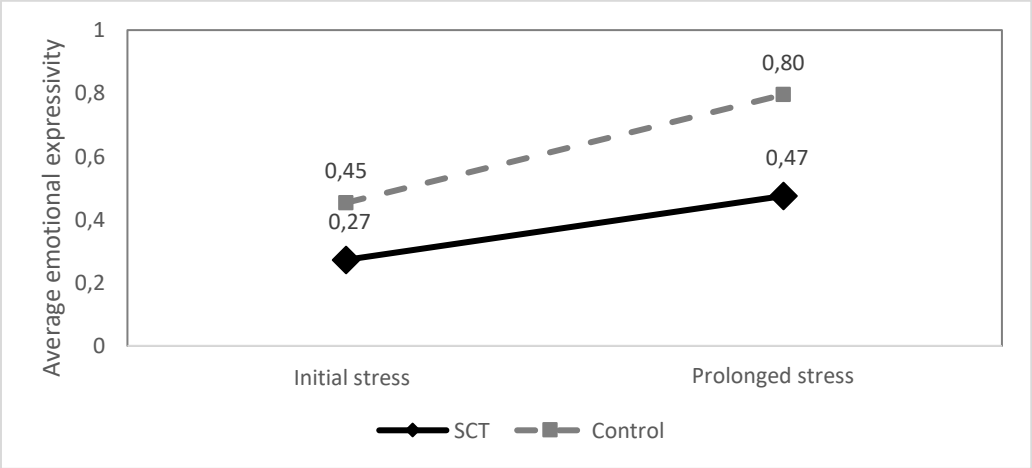
To examine whether the vulnerabilities in physiological reactivity and recovery were present across all ages in children with SCT, bias-corrected bootstrapping analyses (PROCESS) were conducted for the stress arousal response (from initial to prolonged stress) and the recovery arousal response (from prolonged stress to extended recovery). No significant moderation effect of child's age was found for neither the stress response (b = -.07, SE = .92, t = -.07, p = .940, 95% confidence interval = -1.88, 1.75) nor the recovery response (b = 1.14, SE = 1.16, t = .98, p = .328, 95% confidence interval = -1.15, 3.43).

Emotional Expression in Response to Stressful Event

A GLM repeated measure analysis revealed a significant main effect of Task (F(1, 138) = 14.802, p < .001, eta = .097) and a significant effect of Group (F(1,138) = 4.591, p < .05, eta = .032). The interaction effect Task * Group was non-significant (F(1,138) = .929, p = .337). In controls, there was a significant increase in emotional expressivity from the initial to the prolonged stress phase of the task (also see Figure 2). This was also present for children with SCT, but significantly lower compared to the controls.

Figure 2

Average Expression of Emotions During Stress Phases of the Unpredictable Toy Task for Sex Chromosome Trisomies (SCT) Group and Control Group



Role of Age in Emotional Expressivity

To examine whether the vulnerabilities in the expression of emotions were present across all ages in children with SCT, bias-corrected bootstrapping analyses (PROCESS) were conducted. No significant moderation effect of child's age was found for emotional expressivity (b = -.04, SE = .19, t = -.21, p = .833, 95% confidence interval = -.42, .34).

Concordance Between Reactivity and Expressivity

The results from the correlation analyses, performed in each research group separately, revealed significant relationships between the physiological arousal response and the total amount of emotional expressivity, during the stress phase. In controls, there was a significantly strong, positive correlation between arousal response and emotional expressivity (r = .728, p < .001). Although a significantly positive correlation was also found in the SCT group (r = .390, p < .01), this was significantly weaker in comparison to the control group, as shown by Fisher r-to-Z-transformation (z = -2.783, p < .01). In other words, the concordance between arousal reactivity and emotional expressivity was significantly lower in the SCT group as compared to controls.

The Role of Karyotype, Ascertainment Bias, and Recruitment Site

Karyotype

To examine whether the emotion vulnerabilities were present across all three karyotypes of SCT, a MANOVA was performed with karyotype as independent variable, and the three main outcome parameters (stress arousal response, recovery arousal response, and emotional expressivity) as dependent variables. There were no significant differences between the three karyotypes (Pillai's trace = .155, F(6,100) = 1.403, p = .221): boys and girls with an extra X or Y chromosome showed similar patterns in the area of psychophysiological and emotional expressive outcomes.

Ascertainment Bias

To examine whether recruitment strategy was relevant to the increased risk for emotion vulnerabilities, a MANOVA was performed with recruitment strategy within the SCT group (prospective follow-up, information seeking parents, clinically referred cases) as independent variable and the three main outcome parameters (stress arousal response, recovery response, and emotional expressivity) as dependent variables. There were no significant group differences (Pillai's trace=.148, F(6,100)=1.336, p=.248): how children where ascertained for enrollment in the study did not appear to affect the degree of psychophysiological and emotional expressive outcomes.

Recruitment-Site

To examine whether study site was related to emotion vulnerabilities, a MANOVA was performed with study site (the Netherlands, the United States of America) as the independent variable and the three main outcome parameters (stress arousal response, recovery response, and emotional expressivity) as dependent variables. There were no significant group differences (Pillai's trace = .089, $F(6,50) = 1.629$, $p = .194$): children from the United States and The Netherlands showed similar vulnerabilities in the area of psychophysiological and emotional expressive outcomes.

Discussion

The present study examined emotional reactivity and expressivity in response to a stress evoking event in young children with an extra X or Y chromosome, compared to controls. Key to this study was the examination of both physiological components (e.g., affective arousal in heart rate) and observational behavioral components (facial and bodily expressivity) of the emotional system. By studying both parameters with sensitive and objective techniques, we aimed to understand the possible differential deficits and concordance in emotional processing in a young sample of genetically 'at-risk' children. The key finding of the current study is that differential reactivity and recovery patterns were found for children with an extra sex chromosome (SCT) compared to controls. They not only showed a significantly lower arousal response (indicating a blunted response), but also their recovery took longer compared to controls who showed an immediate recovery response. In addition to emotional arousal, we found that children with SCT showed less emotional expressivity during both initial and prolonged stress. Furthermore, our study revealed less interplay between the physiological and behavioral components of the emotion response: children with SCT showed a significantly lower concordance between emotional arousal and expressivity compared to controls when faced with an unexpected (stressful) event. Taken together, these results provide the first evidence of significant vulnerabilities in the responsiveness and recovery of the emotional system of young children with SCT, which may be one of the key mechanisms underlying problems in social adaptive functioning.

Discordance between expression and arousal has not been described elsewhere in the literature on SCT, making our study the first to describe these results in this population. However, in other clinical groups also known for social-emotional difficulties, such children with autism spectrum disorders (ASD), discordance between arousal and expression has also been described (Zantinge et al, 2018). In hypothesizing about the nature of these differences in the affective system of children with SCT, it could be helpful to examine the underlying neurological networks in the brain that are thought to be involved in processing emotional information. For instance, numerous neural brain regions play some role in the emotion system, including subcortical areas such as the amygdala (Costafreda et al., 2008) as well as a set of cortical regions, including the anterior insula and dorsal anterior cingulate (Murphy et al., 2003). Interestingly, neuroimaging findings in SCT (specifically 47,XXY) show consistent neuro-anatomical and functional differences in these areas, including reduced grey-matter volume in both insula and temporal regions, including the amygdala and hippocampus (Hong & Reiss, 2014), suggesting that the physiological and behavioral vulnerabilities are likely anchored in (early) brain maturation. Further support for this early link between genes, brain, and behavior also comes from our finding that age was no significant contributor to physiological arousal or emotional expressivity. Average deviations in emotional processing were present across all ages in children with SCT, even as early as 12 months old. Our results match with those of other studies that also found early signs of an at-risk development in a subgroup of (although not all) children with SCT (Bouw, Swaab, Tartaglia, Cordeiro, et al., 2022; Kuiper et al., 2021; Urbanus, Swaab, Tartaglia, Boada, et al., 2022; Zampini et al., 2021).

To the best of our knowledge, there are only three other studies to date that examined physiological arousal in individuals with SCT, for which the results vary. The first showed increased skin conductance levels in response to social information (emotion-evoking clips) compared to controls, in adults with 47,XXY (van Rijn, Barendse, et al., 2014). The second study (Bizzell et al., 2020) compared healthy controls to school-aged children with 47,XYY with and without an autism spectrum disorder, but found no significant groups differences in their arousal responses to sensory

challenges (i.e., ambulance siren). The third study (Urbanus et al., n.d.), that included the same cohort of children as the current study, found a blunted physiological arousal response in response to social bids using video clips. Differences in these findings might relate to the nature of the stressor (e.g., social, non-social, neutral) and the context in which the arousal is measured (Stifter et al., 1989). The current study was designed to measure emotional reactivity without the possible interference of social interaction aspects, by introducing a stressor that did not require interaction with a second person. Comparing the results from the aforementioned studies to ours suggests that children with SCT might show a blunted arousal response in terms of their own emotions, a typical response to neutral stressors, and an increased arousal response responding to emotions of others. Replication of the current results as well as future studies that examine the influence of the nature of the stressors and the longitudinal pathway of arousal are needed to provide clarity. Nevertheless, our results on reduced emotional expressivity fit with how some young children with SCT are described on a behavioral level, including decreased assertiveness and more reserved, withdrawn, and shy behavior (Otter et al., 2010; Ratcliffe et al., 1990; Ross et al., 2012; van Rijn & Swaab, 2015). Similarly, our results match with other studies that found deviations in emotional expressivity, including difficulties in expressing negative emotions to others (van Rijn et al., 2008), regulating their emotions (van Rijn & Swaab, 2020), and identifying and verbalizing their emotions (Van Rijn et al., 2006).

The current results are of significant additive value to our current knowledge of mechanisms driving the risk for adaptive behavior problems of children with SCT. Traditionally, emotional and behavioral difficulties with SCT have been explained by deficits in information processing skills, including language difficulties and executive function difficulties (for a review see van Rijn, 2019). Based on our data, we offer another important underlying mechanism for explaining the clinical variety so often described in SCT: that of the emotion system. Understanding how vulnerabilities in the emotional system could impact adaptive functioning and behavior is key. Our results show that the signaling function of emotions, indicating that a situation is relevant and requires attention,

works differently in children with SCT. These children appear less likely to signal challenging situations as potentially relevant whilst needing a longer recovery period from the same event. They show a reduced behavioral response to the situation, in terms of emotional expressiveness. The cohesion between these two components is less strong than in typical development, which makes communication of the 'internal state' to the outside world more difficult. In other words, what happens on the inside (arousal) does not match with what happens on the outside (expression). In terms of day-to-day interaction, one can imagine that a disconcordance confuses a care-taker with respect to what a child needs in a specific situation, significantly hindering their adequate involvement in the child's development and functioning. An optimal amount of emotional reactivity is needed for a child to be an active participant in day-to-day life: not too much, given that leads to continuous overwhelming experience of emotions, but also not too little, given that leads to no adequate behavioral activation (passivity). Having just the right amount of reactivity and expressivity helps individuals in their interaction with others by supporting communication of their inner state: it enables actively seeking out those situations that are good and avoiding those that are harmful (Gross, 2013).

When the signaling function of the emotional system does not work properly, a child cannot rely on its automatic function, which makes it difficult for the child to respond automatically and intuitively to changes in his or her environment (Gross, 2013). Organizing behavior so that it is adaptive to the demands of the situation at hand is thus impacted. This impaired emotional system could be an explanation on why children with SCT may experience difficulties in showing appropriate social and emotional behavior. Being human, most of our interactions and functioning take place within a highly complex and social world and well-regulated emotions help us to adaptively respond to changes in our social environment (van Rijn et al., 2012). Evidence for this hypothesis also comes from studies in typically developing children that show that inadequate emotional reactivity has been linked to behavioral problems (T. Beauchaine, 2001; Boyce et al., 2001). Discordance of arousal and expressivity is associated with later increased depressive symptoms and lower well-being (Mauss et

al., 2011). Future studies should examine the link between emotion reactivity and responsivity and behavioral problems later in life in children with SCT, implementing a longitudinal design that goes beyond the scope of the current study. Nonetheless, our data provides the initial evidence that SCT can impact the pattern of reactivity and responsivity of the emotion system, putting these children at an early risk for socio-emotional developmental difficulties.

Strengths of our study include the use of sensitive and objective techniques, such as psychophysiology, in a young sample of predominantly prenatally diagnosed children with SCT. Studying children with a genetic disposition that can be diagnosed prenatally provides a unique opportunity to examine developmental genetic-behavioral-pathways, implementing a prospective approach that goes beyond describing problematic behavior. Instead it focuses on identifying early markers of “at risk” development that could guide early interventions to minimize future adverse outcomes. For clinical practice, our results are of significant value as well. Caregivers and professionals working with children with SCT should be aware of the differential aspects of the emotion system that could contribute to developmental vulnerabilities in early childhood. These children could benefit from orienting-supported interventions (such as guiding children’s attention to those situations that could be relevant) as well as help in expressing emotions in an adaptive way (such as mirroring or verbalizing emotional experiences). In addition, given children with SCT showed an (overall) prolonged reactivity to stress, they might need more time recovering to a “regulated” baseline state when faced with challenges: more than they might display in their behavior. Helping caregivers be aware of possible discordance in children with SCTs emotional expression may help them better support their interactions and responses to novelty.

Several factors limit the impact of the findings from the current study, for which related suggestions for future research are given. First, our study included no indices of arousal other than heart rate and observed expressive behavior in response to a stress-inducing stimulus. A range of different types of stimuli (including both positive and negative, social and non-social loading stimuli) should be explored further as well as other indices of the autonomic nervous system (ANS) including

heart rate variability and information on the parasympathetic branch of the ANS (Benevides & Lane, 2015). Secondly, the current study did not include any cognitive measures or information on emotion regulation strategies. We did not examine how children dealt with the challenging situations in terms of emotion regulation strategies and how it relates to social, behavioral, and emotional functioning in day-to-day life. It would be interesting to examine the predictive value of emotion reactivity and its accompanying emotion regulation strategies over time, to provide insight in the longitudinal relationships of the emotion system and later developmental difficulties, as well as to guide intervention strategies. Whilst our study is of cross-sectional nature and our results showed that age was not a significant contributing factor to emotional reactivity nor responsivity, longitudinal studies are needed to support the developmental impact in individuals with SCT over time and its relation to neurodevelopmental disorders and psychopathology, including but not limited to ADHD and ASD. Further, these study results spark interest into how the genetic differences lead to differences in neurobiological responses, as well as how psychosocial and/or pharmalogical interventions targeting emotional regulation may affect behavioral responses in children with SCT. This line of research could also include the study of the (specific) genetic influence of additional X and Y chromosomes on the social-emotional phenotype, examining differences between different karyotypes as well as comparisons with other (non-sex chromosomal) trisomies.

In conclusion, our findings show early disturbances in the emotion system of very young children with SCT, in terms of blunted and prolonged emotional reactivity and a reduced emotional expressivity in response to stress. We propose that the emotion system could be an important underlying mechanism in explaining the heterogeneity and variability in developmental outcomes so often described in individuals with SCT, given the significant impact emotions have on adaptive day-to-day functioning. The current findings are important for improvement of clinical care in individuals with SCT, given that increased awareness of the discordance between expression and arousal can be meaningful in psycho-education and intervention strategies of individuals with SCT.

Appendix A

Figure A1

Professionally Edited Photo of the Unpredictable Mechanical Toy Task.

Note. This photo was not part of the original published manuscript and was originally made for PR purposes of the TRIXY Early Childhood Study. Photo: ARNICK.nl, printed with permission.

CHAPTER 5

(Not) getting what you want:

Frustration and emotion regulation in

children with sex chromosome trisomies

Submitted upon request for publication as:

Kuiper, K. C., Swaab, H., Tartaglia, N., Cordeiro, L., & van Rijn, S. (2022). (Not) getting what you want: Frustration and emotion regulation in children with sex chromosome trisomies*, Endocrine Connections.*

Abstract

The presence of an additional X or Y chromosome (sex chromosome trisomies, SCT) is associated with an increased risk for neurodevelopmental difficulties, including socio-emotional problems, across the life-span. Studying emotion regulation in early childhood in children with SCT could signal deviations in emotional development that serve as risk markers to guide clinical care. This study explored the presence and variety of emotion regulation strategies in XX SCT children and XX population-based controls, aged 1 to 7 years, during a blocked-goal event in which physiological (heart rate) and observational data (behavioral responses) were collected. Children with SCT showed more difficulties regulating their emotions as compared to typically developing children, had a more limited range of behavioral strategies to implement, and tended to rely longer on inefficient strategies with increasing age. The field of practice needs to be made aware of these early disturbances in emotion regulation in SCT, which potentially lay the fundamental groundwork for socio-emotional problems, given the significant impact of emotion regulation on childhood and adult mental health outcomes, including school readiness and psychopathology. These results help to design tailored interventions to reduce the impact of the additional sex chromosome on social and adaptive functioning.

(Not) Getting What You Want: Frustration and Emotion Regulation in Children with Sex Chromosome Trisomies

One in every 1000 children are born with an additional X or Y chromosome, resulting in a trisomy of the sex chromosomes (SCT, Berglund et al., 2019; Groth et al., 2013). Individuals with SCT are at risk for a wide range of neurodevelopmental difficulties, including behavioral, social, and cognitive problems, that can be present across the life span (for review see: van Rijn, 2019). There is growing support that emotion regulation difficulties are also a serious concern for the SCT population (Tartaglia et al., 2010; van Rijn, Stockmann, et al., 2014; Visootsak & Graham Jr, 2009). **Emotion regulation** refers to all processes that influence the occurrence, intensity, duration, and expression of emotions (Gross, 2013). The ability to regulate emotions is a crucial developmental milestone for children to achieve, in order to adaptively function in our complex social world (Denham et al., 2003). Emotions provide us with key information on how to perceive the world around us (information-oriented), how to accomplish our goals (goal-oriented), and how to respond adaptively to complex situations (action-oriented)(Thompson, 1994). It is thus not surprising that the ability to regulate emotions in early childhood is associated with adaptive outcomes in multiple domains, including school readiness (Blair & Razza, 2007), better social skills (Eisenberg et al., 2010) and fewer externalizing problems (Olson et al., 2005). Whilst individuals with SCT are at significant risk for developing psychopathology, both internalizing and externalizing (van Rijn, 2019), empirical studies on emotion regulation in young children with SCT are scarce. There is a need for more knowledge on emotion (dys)regulation and types of regulatory strategies children with SCT tend to employ in challenging situations. This information could be useful in designing more tailored interventions targeting emotion regulation strategies and to reduce the impact of the additional sex chromosome on social and adaptive functioning throughout the lifespan.

Emotion regulation manifests across multiple systems, including physiological changes, subjective experiences, and behavioral responses (Tracy, 2014). Studies showed that adolescents and adults with SCT can have difficulties in one or more of those systems. To start, emotional reactivity, i.e., the tendency to experience emotional arousal to an event (Rothbart et al., 1981), appears

different in SCT compared to controls. Van Rijn and colleagues (2014) found that the affective arousal (in terms of skin conductance levels) in response to emotion evoking video clips was overall increased in adult men with 47,XXY, compared to controls. This is in line with self-reported data, where men with 47,XXY also described themselves as being more easily aroused by emotion-evoking situations than peers (Van Rijn et al., 2006). In addition to emotional reactivity difficulties, having an additional sex chromosome has also been associated with the use of atypical emotion regulation strategies in adult men (van Rijn & Swaab, 2020), including increased expression of emotions, avoiding, distraction seeking, and passive coping. Furthermore, emotional outbursts (Visootsak & Graham Jr, 2009), affective problems (van Rijn, Stockmann, et al., 2014), and depressive symptoms (Tartaglia et al., 2010) are commonly present in SCT in the age range from school-aged children to adulthood.

Studies thus far provided key information on emotional regulation and its difficulties in school-aged children, adolescents, and adults with SCT. However, knowledge of early development is scarcely available. Because of technological advances in prenatal testing, studies on very young children with SCT become more accessible (Tartaglia et al., 2020). Recently, it was found that deviations in emotional reactivity can be present from early toddlerhood on (Kuiper et al., in press): when faced with an unexpected event (e.g., a moving and sound-producing robot), children with SCT show a blunted affective arousal (in terms of heart rate) compared to non-clinical controls. Moreover, their physiological response was less predictive of a coherent behavioral response (in terms of facial emotional expressions). In addition, Urbanus and colleagues (2020) showed difficulties with overall social–emotional functioning, already present in 1-year-olds with SCT, and elevated scores persistent across the age span of 1 to 5 years old. Affective, pervasive developmental problems, anxiety, attention deficit, and oppositional defiant behaviors were already part of their social-emotional profile. Thus, there is a great need to further understand the developmental processes of emotion regulation and dysregulation in children with SCT. What remains unknown is what young children with SCT explicitly do in terms of regulating their emotions: how do they

organize their behavior when emotions arise? Are these children able to adjust their behavior accordingly so that they can acquire and accomplish their goal, despite arising emotions? The current study aims to provide the answers to these questions.

In studying emotion regulation in young children with SCT, it is important to take into account the developmental process of emotion regulation. Early childhood is characterized by immense progress on multiple developmental domains, including that of emotional control. Whereas early forms of regulation are mainly supported by caregivers in the first year of life (Crockenberg & Leerkes, 2004) and appear more automatic (e.g., avoidance and self-soothing), around the age of 2 years old children develop the ability to control their attention to use it in service of emotion regulation and show more volitional forms of regulation (Posner & Rothbart, 2000). Amongst others, processes that support this are the increase of physical and behavioral opportunities (e.g., moving away from unpleasant situations) as well as developing frontal cortical networks associated with attentional control. As such, the development of emotion regulation in infancy and early childhood can also be seen as a stepping stone through which children attempt to regulate their emotions through behavioral strategies, with the prospect that such regulation will also set the stage for the development of other higher order self-regulation abilities, such as executive functions (Blair & Ursache, 2011). In SCT, a couple of studies have provided initial evidence that the developmental trajectory of children with SCT seems to be different compared to nonclinical controls (Urbanus, Swaab, et al., 2020; Kuiper, et al., 2022) with more pronounced behavioral problems in late early childhood compared to toddlerhood, thus highlighting the importance on studying children with SCT in infancy and preschool period.

The results of the current study are of significant importance. First, it provides information on early markers that could signal at-risk development in young children with SCT. Furthermore, it could help develop specific tools for early and preventive intervention aimed at emotion regulation, to reduce the impact of the additional sex chromosome on social and adaptive functioning throughout the lifespan. This study will examine the presence and variety of emotion regulation skills within a

genetically vulnerable group (SCT) compared to a general population sample. A specific focus is on how children with SCT cope with arising emotions in the context of a frustrating situation compared to controls and whether developmental aspects such as age are also relevant. Of interest to the current study is not the sole use of a single strategy, but rather the variety of emotion regulation strategies that are essential to adequately influence the occurrence, intensity, duration, and expression of emotions (Gross, 2013).

Methods

Participants

The current study is part of a larger international study, the TRIXY Early Childhood Study, based at Leiden University in the Netherlands. The TRIXY Early Childhood Study investigates the social, emotional, and behavioral development of young children with a trisomy of the X/Y chromosomes (TRIXY). Children aged 1 to 7 years were recruited from two sites: the Centre of Expertise for Trisomy of the X and Y chromosomes (TRIXY) in the Netherlands and the eXtraordinarY Kids Clinic in Developmental Pediatrics at the Children's Hospital Colorado (CHCO) in the United States (USA). TRIXY recruited children from Dutch-speaking countries in Western Europe (*n* = 39) and the eXtraordinarY Kids Clinic recruited children from across the USA (*n* = 36). Recruitment of SCT children took place with the help of clinical genetics departments, pediatricians, and national advocacy or support groups for (parents of) individuals with SCT using recruitment flyers and postings on the internet and social media. Based on how SCT children enrolled into the study, three different inclusion trajectories could be identified (see Table 1). Children in the control group were recruited from day care centers, public institutions, and elementary schools from the western part of the Netherlands. To be included in the study, both parents and children needed to be Dutch- or English-speaking and children needed to be free of (history of) head injuries, severely impaired hearing or sight, and/or colour-blindness.

Table 1

Demographic Characteristics of the Sex Chromosome Trisomies (SCT) and Control Group

	SCT	**Controls**	**Group differences**
	n = 75	*n* = 81	
Age in years – *M (*SD)	3.82 (1.90)	3.82 (1.60)	$t(154) = .01$, $p = .992$
Gender	M = 49, F = 26	M = 35, F = 46	$X^2 (1) = 7.669$, p **< .01**
Parental education level – median (range)	6 (4 – 7)	6 (2 – 7)	$p = .794$
Karyotype		N/A	
XXX	26		
XXY	39		
XYY	10		
Recruitment strategy - *n* (%)			
Information-seeking parents	38 (50.7%)		
Prospective follow-up	25 (33.3%)		
Clinically referred	12 (16.0%)		

In the current study, 75 children with SCT and 81 age matched population-based controls participated (Table 1). The SCT group consisted of 26 girls with 47,XXX, 39 boys with 47,XXY, and 10 boys with 47,XYY. More than half (56%, *n* = 42) had a prenatal diagnosis (i.e., because of [routine] prenatal screening, abnormal ultrasound findings, or advanced maternal age) next to 33 children (44%) who had a postnatal diagnosis (i.e., because of developmental delay, physical and/or growth problems, or medical concerns). Confirmation of trisomy in at least 80% of the cells was provided by standard karyotyping. Parents were asked to present a copy of the karyotyping report of the child that was provided by their clinician at time of diagnosis. Children from the control group were not subjected to genetic screening. Given the prevalence of SCT (1 in 1000) in the general population, we decided that the burn of blood draw for testing for SCT in our control group outweighed its potential

utility. We reviewed the possible risk of having a child with undiagnosed SCT in our control group minimal and acceptable. Parental education level was assessed according to the Hollingshead criteria and ranged from category 1 (no formal education) to 7 (graduate professional training) (Hollingshead, 1975). When the child was raised by two parents (96% of the children in the current sample), educational level was averaged over both parents. Parental education level was comparable over research groups, as well as age (also see Table 1).

Ethics and Procedure of the Assessment

Both the Ethical Committee of the Leiden University Medical Centre in the Netherlands and the Colorado Multiple Institutional Review Board (COMIRB) in the USA approved the TRIXY Early Childhood Study. Researchers from Leiden University supervised project and data-management, including training of experimenters and processing of data. Primary caregivers of all participating children signed a written informed consent prior to the research visit. Additionally, caregivers received a visual information brochure and a copy set of the electrodes for the physiological assessment to help children prepare. Research visits took place in a quiet stimuli-low room either at the university or the family's home, using written protocols detailing all procedures and verbal instructions to standardize assessments across countries. During assessment children had additional time to familiarize before and after the electrodes were applied by playing an age-appropriate game, while seated in a car seat to have a stable and framed position suited for physiological measurement.

Physiological Arousal

Two electrodes were attached at the top center of the chest (10 centimeters below the suprasternal notch) and the bottom left of the ribs (10 centimeters above the bottom of the rib cage). Heart rate was recorded continuously during baseline and locked box task with AcqKnowledge (version 5.0.2, BIOPAC Systems Inc.). An Electrocardiogram amplifier (ECG100C) and a BIOPAC data acquisition system (MP150 Windows) with a sampling rate of 1.000 Hz were used for recording. In AcqKnowledge a 0.5 Hz highpass filter and 50 Hz notch filter were applied to stabilize the ECG signal. Recorded physiological data was further processed by inspecting the detected R peaks in PhysioData

Toolbox version 0.5.0 (Sjak-Shie, 2020). Motion artifacts were visually identified and excluded from the data. Heart rate data (beats per minute: BPM) were summarized in 30-second epochs in concordance with the behavioral data. To establish a baseline heart rate, children watched a 3 minute video of a fish tank, which has been shown to be an adequate measure of resting state (Piferi et al., 2000). Heart rate (in BPM) over the course of the video was analyzed in epochs of 30 seconds each and the epoch in which children had the lowest heart rate was identified as representing resting state. This was done on group level, for the control group and the SCT group separately.

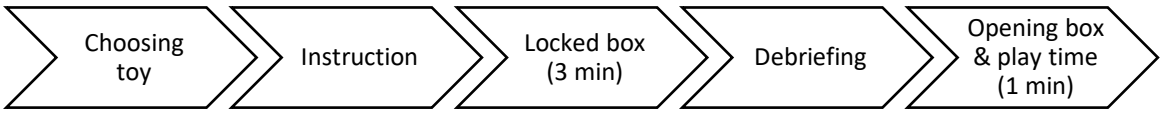
Frustrating Task (Blocked-Goal Paradigm)

The locked box task from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith et al., 1999) was selected to elicit behavioral and emotional responses, including frustration, given that it incorporates a blocked-goal paradigm. The experimenter placed three age-appropriate attractive toys in front of the child but outside the child's reach. The child was encouraged to choose one preferred toy, which was then placed in a transparent locked box. The unwanted toys were removed from sight and children were instructed to try to acquire the selected toy in any way they can (although they were unable to succeed given that the box is locked). The experimenter left the child alone to deal with the box. Following 3 minutes of manipulation (i.e., frustration phase), the experimenter returned to the child and opened the box, after which the child was encouraged to play with the toy for 1 minute (i.e., recovery phase). This paradigm deliberately prevents children from playing with a preferred toy and thus provides the opportunity to examine how children naturally cope with their emotions and organize their behavior when goals are blocked (i.e., to acquire a toy). The procedure of the task followed the Lab-TAB manual (see Figure 1 for a timeline) with one exception. For the current study, we used two boxes depending on the child's age: a locked box with keys for children aged 3 and up (as described in the Lab-TAB manual) and a modified box for the youngest age group (1 to 2 years old) in which the lid was not locked with keys, but strongly secured with additional grip. This adjustment was necessary to prevent bias due to differences in motor skills, given that not all children before the age of 2 already have the developed

fine motor skills essential to use and open a lock with keys, whereas most children have developed gross motor skills essential to remove a lid. Caregivers were instructed to sit in the back of the room out of direct sight, filling out questionnaires or reading magazines, and to remain as uninvolved as possible while displaying a neutral face. Only during recovery, caregivers were allowed to sooth and comfort their child, but the child stayed in the car seat for the remainder of play time to stabilize the physiological recovery assessment. The entire procedure was videotaped from two angles.

Figure 1

Timeline Locked Box Task



Note. Original locked box task described in Lab-TAB (Goldsmith et al., 1999).

Data from the locked box were analyzed in 30 second epochs. Of the total of 180 seconds (3 minutes), the first 30 seconds were discarded to allow for a build-up of frustration (i.e., children first need to try the most obvious ways to find out that the box is indeed locked). The majority of the children were able to complete the full 3 minutes (n = 72 of the SCT children and n = 81 of the control children), however some children could not and for example started fidgeting with the electrodes which could have compromised physiological recording. To prevent bias due to the duration of the task, it was decided to use the epochs that included data from all children, resulting in seconds 30 through 120.

Observational Coding of Emotion Coping Strategies

Videos of the locked box task were coded and categorized in 10-second epochs (with sound on) for emotional coping strategies, as described in Jahromi et al. (2012). In total 14 emotion coping strategies were coded in 10-second intervals as either present or absent (scored as 1 or 0). These 14

coping strategies were assessed independently which means that more than one strategy can occur in the same 10-second interval. Strategies were grouped into three categories and their average inter-rater reliability (expressed in Cohen's kappa) were (a) *constructive* strategies (consisting of strategy behaviors such as goal-directed behaviors, orienting to experimenter or parent, and social support seeking); k = .90, (b) *venting* strategies (vocal venting, self-soothing, and self-speech); k = .86, and (c) *avoidance* strategies (avoidance, distraction, and alternate strategies); k = .78. Inter-rater reliability (IRR) was assessed using a two-way mixed, absolute agreement intra-class correlation model (Hallgren, 2012). Six trained independent coders scored all recorded videos. IRR was monitored continuously in regular consensus meetings. Discrepancies were discussed within the team to obtain a final consensus score. Four strategies (disruptive behavior, physical venting, staring, and other-directed comfort-seeking) occurred too infrequent to be included in subsequent behavioral composites or analyses.

Statistical Analyses

As preliminary analyses, to examine whether certain SCT group characteristics were relevant to emotional processing, three MANOVA's were performed with recruitment site, karyotype, and recruitment strategy as (three) independent variables and the two main outcome parameters (including peak arousal response and three emotion regulation strategies) as dependent variables. In addition, to establish that the locked box task was successful in eliciting emotional arousal, a GLM repeated measures analysis was performed with the between subject factor Group (SCT, control) and the within-subjects factor Task (30 seconds baseline heart rate, 90 seconds frustration, and 60 seconds recovery). To further analyze the heart rate pattern over time and potential group differences, tests of within-subjects contrasts were done within the GLM RM analysis. After visual inspection, the highest peak in arousal for each individual was subtracted with baseline heart rate to reflect individual arousal response.

To answer the main research question, a Multivariate Analysis of Variance (MANOVA) with group (SCT, control) as fixed factor and coping strategies (Constructive, Venting, and Avoidance) as

dependent variables was performed. Arousal response was included as a covariate to exclude any interference from individual arousability during the frustrating task. The moderating effect of age was assessed using PROCESS, a bootstrapping, nonparametric resampling procedure (Hayes, 2009). Bootstrapping analysis with 5000 resamples was done to test for a significant moderating effect using the SPSS macro developed by Hayes (2017). Outcome variables and moderator variable (i.e. child's age) were centered, peak arousal response was included in the analysis as a covariate to control for individual arousability. In this analysis, the moderation effect is significant if the 95% bias corrected confidence interval for the moderator effect does not include zero.

For correlation analyses with age, Pearson's product moment correlation coefficient was used. Level of significance was set at $p = .05$. For all significant effects, Cohen's *d* and η^2 addressed effect size (.2 = small effect; .5 = medium effect; .8 = strong effect, Cohen, 1977).

Results

Preliminary Analyses

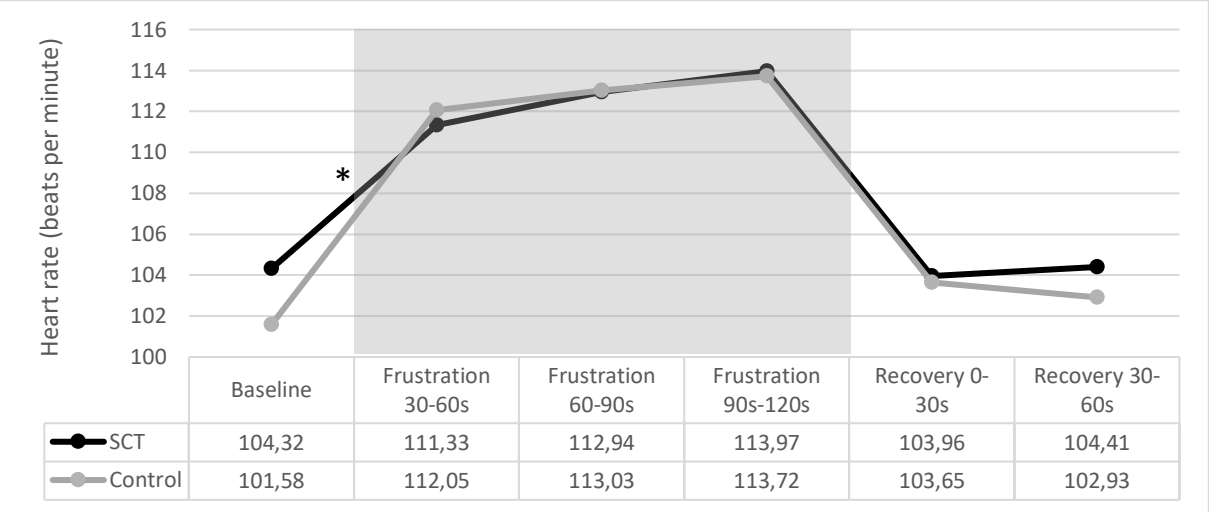
Results from the preliminary analyses revealed no evidence of significant differences in the SCT group regarding recruitment site, karyotype, and recruitment strategy on psychophysiological and emotion regulation outcomes. Children with SCT from the Netherlands had similar results as the children from the United States on the main emotion outcome parameters (including arousal response and three emotion regulation strategies, Pillai's trace = .108, $F(4,70) = 2.120$, $p = .087$). In addition, which karyotype a child carried (XXX, XXY, and XYY) had no influence on the main outcome parameters (Pillai's trace = .058, $F(8,140) = .522$, $p = .838$). Finally, how children with SCT enrolled in the study (prospective follow-up, information seeking parents, and clinically referred cases) had no significant effect (Pillai's trace = .028, $F(8,140) = .245$, $p = .981$). These results provided the support to pool SCT children from various recruitment sites, karyotypes, and recruitment strategies together and include the total group of SCT children in further analyses.

Efficacy of the Arousal-Inducing Paradigm

Prior to examining emotion regulation strategies, analyses were performed to establish whether the locked box task was indeed successful in inducing emotional arousal. First, in terms of baseline heart rate, there was no significant difference between children with SCT (M 104.32, SD 17.27) and children from the control group (M 101.67, SD 14.10) (t(154) = 1.050, p = .295). With regards to the pattern of arousal response (corrected for baseline heart rate), a GLM repeated measures analysis was performed and revealed a significant main effect of Task (F(5,149) = 57.105, p < .001, np^2 = .657), no main effect for Group (F(1,101) = .059, p = .808), and no interaction effect (F(5,149) = 1.623, p = .157). These results showed no significant difference in the pattern of arousal response across groups during the locked box task (also see Figure 2). Thus, the locked box task was successful in evoking an emotional response in both groups, laying the fundamental ground to examine emotion regulation strategies in the context of frustration. Noteworthy: tests of within subjects contrasts revealed similar increases and decreases for both groups during the full locked box task, with one exception. There was a significant interaction Task x Group effect from baseline to the frustration phase (F(1,153) = 6.586, p < .02, np^2 = .041), indicating that the increase in initial arousal response was significantly stronger for control children than for SCT children. However, there were no other significant interaction effects during any of the other time-points of the frustrating task. Finally, all children (both SCT and controls) were able to return to resting state during recovery.

Figure 2

Heart Rate Pattern During Locked Box Task for SCT Group and Control Group



*Note. T*he initial 30 seconds from the frustrating task were excluded from analysis to allow for the build-up of frustration. SCT: Sex Chromosome Trisomies.

Behavioral Regulation: Emotion Regulation Strategies

The main research objective was to examine the presence and variety in emotion regulation strategies in the context of frustration and compare group differences between SCT and controls. Examination of the descriptives revealed that during the locked box task, all children (both SCT and controls) most often showed Constructive Strategies followed by Venting Strategies and then Avoidance Strategies (Table 2). To compare group differences between SCT and controls, peak arousal response was included as a covariate in a MANCOVA to account for individual differences in arousability, with the three emotion coping strategies (Constructive, Venting, and Avoidance) as dependent variables and Group (SCT, control) as fixed factor. The results revealed a significant main effect for arousal response ($F(3,151) = 7.345$, $p < .001$), $np^2 = .127$) and Group during the locked box task ($F(3,151) = 3.253$, $p < .05$; Pillai's trace = .061, $\eta p^2 = .061$). When confronted with blocked-goals, children with SCT engaged significantly less in Constructive strategies as compared to children in the

control group, even while correcting for individual arousability. For Venting or Avoidance strategies, there were no significant group differences (also see Table 2 and Figure 3).

Role of Age in Emotion Regulation Strategies

To examine whether group differences in emotion regulation strategies (given individual arousability) were present across all ages in children with SCT, bias-corrected bootstrapping analyses (PROCESS) were conducted. A significant interaction effect of child's age x group was found for Avoidance strategies (b = -.17, SE = .06, t = -2.70, p < 0.01, 95% confidence interval = -.29 and -.05). As shown in Figure 3, the use of avoidance strategies declines with age for both groups, but less rapidly for the SCT group. No significant interaction effect was found for Venting (b = -.07, SE = .11, t = -.61, p = .542, 95% confidence interval = -.29, .15) or Constructive strategies (b = .01, SE = .09, t = .09, p = .931, 95% confidence interval = -.16, 18), also see Figure 4 for a visual representation.

Table 2

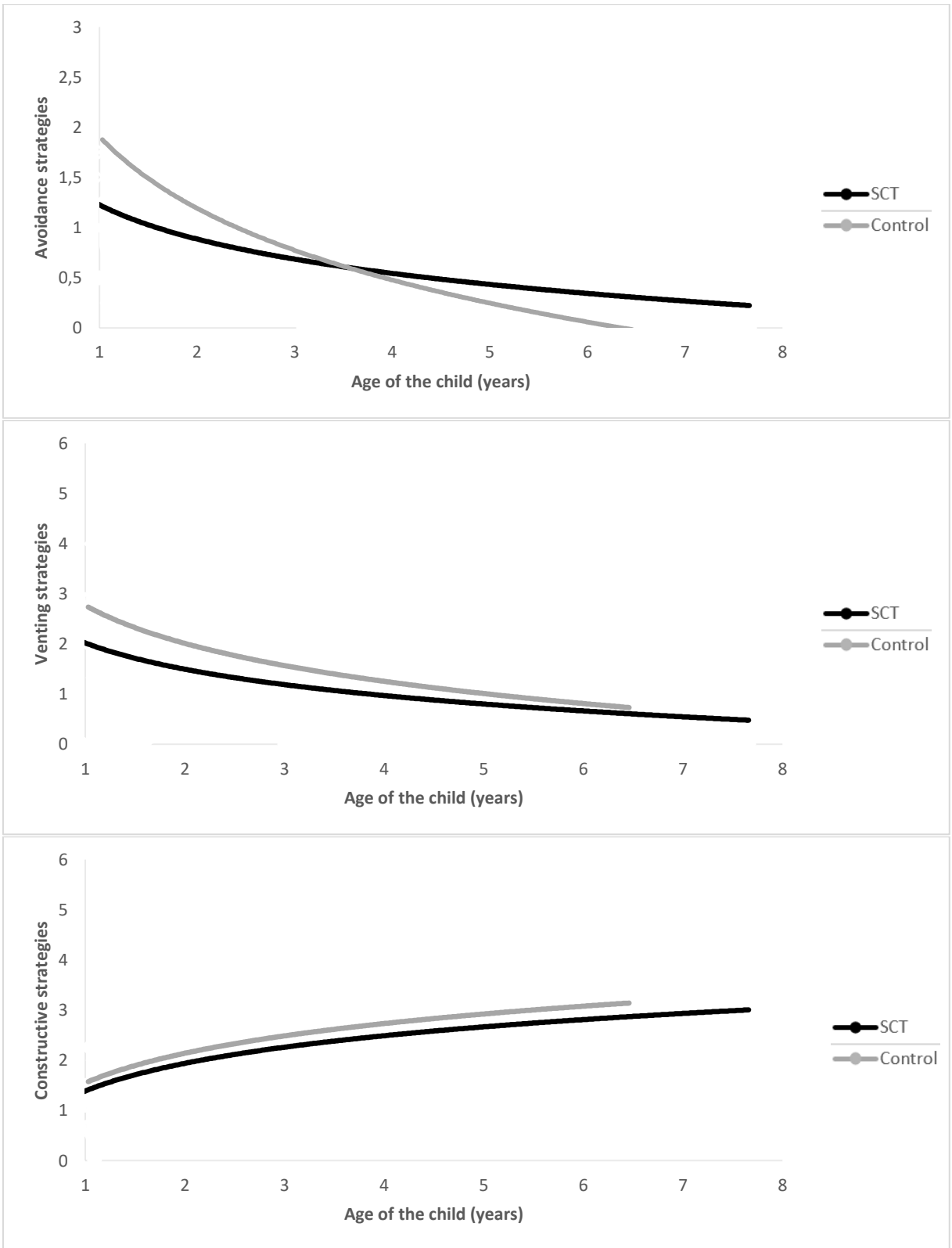
Descriptives and MANCOVA Test Results for Emotion Regulation Strategies

	SCT	Control		
	M (SD)	*M (SD)*	*F*	*p*
Avoidance	0.65 (0.83)	0.65 (0.80)	.006	.936
Venting	1.13 (1.16)	1.49 (1.45)	1.614	.206
Constructive	2.32 (1.11)	2.62 (0.92)	4.443	**.037**

Note. SCT: Sex Chromosome Trisomies.

Figure 4

Scatterplots of Child's Age by Mean Emotion Regulation Strategies Across Research Groups



Discussion

The present study examined emotion regulation processes in young children with a sex chromosomal trisomy (SCT) compared to controls when faced with a frustrating task (blocked-goal paradigm). The results show that while children with SCT experienced a significant increase in arousal response, when blocked in achieving a desired toy, their behavioral response did not follow suit. While controlling for individual arousability, observational measures of behavioral regulation revealed significant differences between groups in those strategies that are most frequently implemented, namely constructive strategies. An interesting developmental effect was also found; whereas avoidance strategies tend to decline with age in typically developing children, this was significantly less so in children with SCT. Furthermore, these results were consistent over karyotypes (XXX, XXY, and XYY), recruitment sites (The Netherlands/the USA), and recruitment strategies, suggesting that emotion regulation is a relevant risk marker in the development of all children with SCT.

The current results indicate that children with SCT may have more difficulties regulating their emotions as compared to typically developing children, may have a more limited range of behavioral strategies to implement, and tend to rely longer on more inefficient strategies with increasing age. To start, one of the key results is that compared to their peers, children with SCT showed less emotion regulation strategies that can be considered constructive. Constructive strategies represent behavioral responses as goal-directed actions and support seeking behavior, in order to achieve a goal at hand. Emotion regulation, in definition, is the ability to organize behavior that could lead to an decrease (or an increase) of negative (or positive) emotions in order to meet the demands of the environment (Gross, 2013). Consequently, these current results indicate that while children with SCT become emotionally affected in the face of frustration, they appear less equipped to regulate their arousal compared to peers: their repertoire of (cognitive) behavioral responses is limited. Furthermore, another key result of the current study is that with older age, children with SCT tend to rely longer on other less volitional forms of regulation, such as avoidance. Avoiding (displeasant

situations) is one of the earliest and automatic forms of emotion regulation, that gradually decreases in use starting at around the age of 18 to 24 months alongside the emergence of other types of regulation strategies (Harman et al., 1997). This shift from automatic to volitional forms of regulation is amongst others associated with the developing (pre)frontal cortical networks during this age period, that at the same time sets the stage for the development of other cognitive functions, including executive functions (Ursache et al., 2013). Taken together with the fact that the genes on X and Y chromosome are densely involved in brain development (Zechner et al., 2001), our results suggests that emotion regulation difficulties might be anchored into early brain maturation of children with SCT. However, it is also important to note here that not all children with SCT were equally impaired and that variety in the use of emotion regulation strategies was also observed. Increasing number of studies are showing that it is not solely the excessive use of maladaptive strategies or the limited use of adaptive strategies that leads to psychological distress and psychopathology, but rather a combination of the two (Braet et al., 2014; Cracco et al., 2015; Schäfer et al., 2017). This suggests that not "one" perfect emotion regulation strategy exist and that rather the variety in strategies, thus the ability to choose from strategies to adapt your behavior to the difficult situations, is truly important for good emotional health.

The current findings fits with behavioral observations of emotion regulation difficulties in daily life, found in children with SCT already from the age of 3 years old (Kuiper, Swaab, Tartaglia, van Buggenhout, et al., 2022; Lee et al., 2011). It is also in line with findings in adults with SCT, that showed atypical emotion regulation strategies in men with Klinefelter (Van Rijn et al., 2006; van Rijn & Swaab, 2020), including difficulties with verbalizing their emotions and higher indices of avoidance, distraction seeking, and passive coping strategies. In addition, the current study add to other findings on psychophysiological reactivity, that suggested that emotional arousal could relate differently to empathic behavior in men with 47,XXY compared to controls (van Rijn, Barendse, et al., 2014) as well as a differential arousal response in young children with SCT (Kuiper et al., 2022). All these findings taken together indicate just how vulnerable individuals with SCT are in their emotional development.

Given that emotion regulation is essential for daily life functioning and an important developmental task in early childhood, our results show that individuals with SCT face significant challenges in dealing with day-to-day conflicts that require children to cope with their emotions adaptively, from an early age on.

Successful emotion regulation in preschool years is associated with social competency, school engagement, and academic performance, in early school years (Robson et al., 2020). On the other hand, emotion regulation is also considered an important process in the etiology and maintenance of different forms of psychopathology (Aldao et al., 2010). It is therefore not surprising that emotion regulation has been acknowledged as a transdiagnostic mechanism in mental health (Insel et al., 2010). Many neurodevelopmental disorders and psychopathology are characterized by difficulties in the emotion regulation system, including mood and affective disorders, autism spectrum disorders, and attention deficit/hyperactivity disorder (American Psychology Association, 2013). From existing literature, it is known that the SCT population is at significantly increased risk for these types of mental health problems (van Rijn, 2019). Studying a genetically vulnerable population such as SCT provides the opportunity to examine gene-brain-behavior pathways of important developmental domains (including emotion regulation) over time. A prenatal diagnosis of a genetic condition such as SCT helps to identify populations at risk, before any significant problems or psychopathology emerge. This information is crucial to understand how neurocognitive skills and processes develop over time and contribute to potential psychopathology. Furthermore, it could reveal risk markers in early development that can guide early (preventive) intervention, not limited to only those individuals with SCT.

In addition to understanding gene-brain-pathways from a theoretical point of view, these results are also highly relevant to individuals affected with SCT and the professionals working with them and their families. The early signs of emotion regulation dysfunction inform us that children with SCT have difficulties in regulating their emotions, may have a more limited range of behavioral strategies to implement, and tend to rely longer on more inefficient strategies with increasing age. In

supporting their development, emotion regulation may serve as a relevant target for prevention and intervention. Emotion regulation training, especially in young children, should be focused on educating children, their parents/caregivers, and their teachers as well. Children learn emotion regulation skills through observation, adult(parent)-child interactions, and their development is also influenced by the emotional climate in the family (Morris et al., 2007). Training emotion regulation skills in the early years is especially relevant given that the growth in development of emotion regulation skills in early childhood is substantial (Kopp, 1989). Although speculative, our results suggest that it might be helpful to address and teach the use of emotion regulation skills in interventions from a cognitive top-down approach: how to shape your behavior through the use of adaptive emotion regulation strategies in order to control your emotions. Existing programs that incorporates elements of modeling and teaching emotion regulation skills as well as relaxation techniques, that could also be meaningful for the SCT population, are EUREKA in children (Braet & Berking, 2019) or Affect Regulation Training (ART) in adolescents and adults (Berking & Whitley, 2014). Recent developments in (preventive) interventions for individuals with SCT are promising, in terms of improving emotional skills through neurocognitive training. For example, Martin and colleagues (2020) showed that a social management training, that included emotion regulation techniques, led to improvements in emotional stability and self-regulation in adolescents and adults with 47,XXY. Even more promising is the study of Bouw and colleagues (Bouw, Swaab, & van Rijn, 2022) that showed that young children with SCT (before the age of 8 years) can improve their emotion recognition skills through the means of a parent-child at home training program. Concluding, early monitoring on emotion regulation is essential given it allows for the implementation of early (preventive) strategies, which has the potential to influence child development towards more optimal outcomes (Guralnick, 2011).

The study also had some limitations. The current design did not allow for the investigation of interactive dynamics between regulation strategies and subsequent changes in arousal. Also, although the current study showed that the role of age was different for different emotion regulation

strategies, these results are based on a cross-sectional design. Longitudinal studies are needed to further study developmental impact of the extra X or Y chromosome over time, as well as its relation to neurodevelopmental disorders and mental health problems. With regards to direction for future research, emotion regulation (and emotions in general) is considered a complex and multifaceted construct, linking to many child factors, including executive and cognitive functioning and temperament, as well as family context characteristics, including attachment style, childrearing behavior (Morris et al., 2007). Including all relevant factors goes beyond the scope of this study and would require a vastly larger sample. However, future research should try to examine these factors related to emotion regulation, given that it could provide additional markers for early and preventive interventions.

In sum, children with SCT show early deviations in the use of emotion regulation strategies, compared to controls, which could impact their developmental outcomes. Emotion regulation is a valid candidate as a underlying mechanism in explaining the heterogeneity and variabilities in psychopathology and mental health problems, given the significant impact of emotions on adaptive day-to-day functioning. The current findings provide essential information for those involved in care for individuals with an additional X and Y chromosome and help develop specific tools for early and preventive intervention.

Appendix A

Figure A1

Professionally Edited Photo of the Locked Box Task.

Note. This photo was not part of the original submitted manuscript and was originally made for PR purposes of the TRIXY Early Childhood Study. Photo: ARNICK.nl, printed with permission.

CHAPTER 6

Neurocognitive and behavioral development in young children (1-7 years) with sex chromosome trisomy

Submitted on request for publication as:

Van Rijn, S., Kuiper, K.C., Bouw, J.C., Urbanus, E.L., & Swaab, H. (2022). Neurocognitive and behavioral development in young children (1-7 years) with Sex Chromosome Trisomy, *Endocrine Connections.*

Abstract

Investigating sex chromosome trisomies (SCT) may help in understanding neurodevelopmental pathways underlying risk for neurobehavioral problems and psychopathology. Knowledge about the neurobehavioral phenotype is also needed to improve clinical care and preventive intervention for the increasing number of early diagnosed children with the recent introduction of noninvasive prenatal screening. The TRIXY Early Childhood Study is a longitudinal study designed to identify early neurodevelopmental risks in children with sex chromosome trisomy, aged 1 to 7 years. This review summarizes results from the TRIXY Early Childhood Study, focusing on early behavioral symptoms in areas of Autism Spectrum Disorder, Attention-Deficit/Hyperactivity Disorder, and communication disorders, and underlying neurocognitive mechanisms in domains of language, emotion regulation, executive functioning, and social cognition. Behavioral symptoms were assessed through structured behavior observation and parental questionnaires. Neurocognition was measured using performance tests, eye-tracking, and psychophysiological measures of arousal. In total, 209 children aged 1 to 7 years were included: 107 children with SCT (33 XXX, 50 XXY, 24 XYY) and 102 age-matched population controls. Study outcomes showed early behavioral symptoms in young children with SCT, and neurocognitive vulnerabilities, already from an early age onwards. Neurobehavioral and neurocognitive difficulties tended to become more pronounced with increasing age, and were rather robust; independent of specific karyotype, pre/postnatal diagnosis or ascertainment strategy. A more longitudinal perspective on neurodevelopmental 'at risk' pathways is warranted, also including studies assessing effectiveness of targeted early interventions. Neurocognitive markers that signal compromised neurodevelopment may prove to be helpful in this. Focusing on early development of language, social cognition, emotion regulation, and executive functioning may help in uncovering early essential mechanisms of (later) neurobehavioral outcome, allowing for more targeted support and early intervention.

Neurocognitive and Behavioral Development in Young Children (1-7 Years)

with Sex Chromosome Trisomy

About 1 in 650-1000 children are born with a 47,XXY, 47,XXX or 47,XYY chromosomal pattern, as a result of having an extra X or Y chromosome (Bojesen et al., 2003). Knowledge about the neurocognitive and behavioral phenotypes of these sex chromosome trisomies (SCT) remains rather limited in comparison to other chromosome trisomies such as trisomy 21. This is somewhat surprising considering the disproportionate amount of genes on the X chromosomes that have been linked to brain functioning (Zechner et al., 2001) and the reported congruent effects of X- and Y-chromosomes on the proportional size of cortical brain systems involved in adaptive functioning (Raznahan et al., 2016).

However, research has been fueled by an increasing awareness that studying gene-brain-behavior pathways in genetic conditions such as SCT may significantly contribute to our understanding of mechanisms of developmental risk that underlie neurobehavioral psychopathology. It has been proposed that such a bottom-up 'behavioral neurogenetics approach' (Reiss & Dant, 2003) may provide a powerful tool that can complement the top-down study of populations identified based on behavioral classification of psychopathology. An advantage of studying SCT is that the genetic condition can be identified already very early in life through noninvasive prenatal screening testing (NIPT), which offers the opportunity to prospectively study early neurodevelopmental markers of individual differences in neurodevelopmental outcome.

The number of children prenatally diagnosed with SCT is expected to rise rapidly with increasing availability of the NIPT (Gadsbøll et al., 2020; Loughry et al., 2022). This calls for more knowledge about the phenotype of SCT to be able improve counselling, psychoeducation, and clinical care through early support or intervention if needed. There is a gap in knowledge specifically in the neurobehavioral and neurocognitive domain, as traditionally the majority of research studies (about 75%) has focused on the somatic/medical phenotype, with only 25% of the studies focusing on the neurobehavioral phenotype (Pieters et al., 2011). In addition to identifying the range and severity of

neurobehavioral problems that may be seen in SCT, it is also of great importance to have a better understanding of the early underlying cognitive mechanisms of behavior problems. Similar behavioral problems may arise from different underlying information processing dysfunctions in the brain. Knowledge about the cognitive processes that drive behavioral problems in SCT is essential for identifying the nature of developmental vulnerability, as well as the recognition of specific targets for early and preventive intervention, allowing for more tailored mental healthcare.

The TRIXY Early Childhood Study is a longitudinal study designed to identify early neurodevelopmental risks in children with sex chromosome trisomy. Based on studies in adolescents and adults with SCT showing increased risk for social dysfunctioning, neurobehavioral problems and psychopathology, one of the aims of this study was to identify early signs and symptoms in young children with SCT. Focus was on symptoms of neurodevelopmental disorders: Attention-Deficit/Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), and communication disorders. Key to the study was investigating the neurocognitive underpinnings of behavior rather than behavioral symptoms alone. Neurocognitive impairments may serve as sensitive, early predictors of behavioral problems in later life, may function as markers for children with an 'at risk' development, and may provide specific targets for early support and intervention.

Therefore, neurocognitive functioning of children with SCT was extensively studied, with a focus on early development in the age range of 1 to 7 years. Key domains of interest were language/communication, social cognition, emotion regulation, and executive functioning, because 1) these are vulnerable domains identified in studies in adolescents and adults with SCT , 2) that have been found to be key mechanisms driving neurobehavioral problems in adolescents and adults with SCT yet remain largely understudied in children, and 3) these neurocognitive functions are developing at this young age showing individual differences in maturation (Urbanus, van Rijn, et al., 2020; van Rijn, 2019).

The TRIXY Early Childhood Study is based at the TRIXY Center of Expertise at Leiden University, the Netherlands, including a range of (inter)national recruitment and testing sites,

including the Extraordinary Kids Clinic, Children's Hospital Colorado, Denver, USA, directed by dr N. Tartaglia. In total, 107 children with SCT aged 1 to 7 years were recruited with the help of clinical genetic departments, pediatricians, and national advocacy or support groups. All children with SCT had been diagnosed based on standard karyotyping for chromosomal abnormalities, with ≥ 80% of the cells showing SCT. The SCT group included children showing variation in SCT karyotype (XXX, XXY, XYY), time of diagnosis (prenatal, postnatal), or ascertainment/recruitment bias (i.e., the reason for enrollment in research). This variation allowed us to statistically test if specific SCT subgroups were characterized by different risk profiles. Recruitment strategy ('ascertainment bias') included a 'prospective follow-up' subgroup (51%) including children with a prenatal diagnosis who were actively followed over time, an 'information seeking' subgroup (30%) including families looking for information about SCT but without specific concerns of their child's development, and a subgroup of 'clinically referred cases' (19%) including children from families with specific developmental concerns. Within the SCT group, 33 girls with XXX, 50 boys with XXY, and 24 boys with XYY were included. In terms of timing of diagnosis, 67% of the children were prenatally diagnosed, versus 33% postnatally. An age matched non-clinical control group of 102 children (58 girls and 44 boys) was recruited in the Netherlands. For all children exclusion criteria were a history of traumatic brain injury, severely impaired hearing or sight, neurological illness, or colorblindness. As part of the longitudinal design of the study, children were assessed during an initial baseline assessment and a follow-up assessment 12 months later, with a subgroup participating in additional neurocognitive training with a post-intervention follow up.

In this review, we present findings from the TRIXY Early Childhood Study in terms of 1) the broad behavioral profile of young children with SCT aged 1 to 7 years, as well as specific domains of neurocognitive and behavioral functioning: 2) Language and communication, 3) Social cognition, social adaptive behavior and autism spectrum symptoms, and 4) Emotion regulation, executive functioning and symptoms of ADHD.

1. The Broad Behavioral Profile of Young Children with SCT

The social-emotional and behavioral profile of children was assessed with the DSM scales of the child behavior checklist (Achenbach & Rescorla, 2000) and the ages-and-stages social-emotional questionnaire (ASQ-SE-2; (Squires et al., 2015)). The CBCL DSM scales assesses emotional and behavioral problems that were present in the past six months within five different profiles: (1) affective problems (as indication for mood disorders), (2) anxiety problems, (3) pervasive developmental problems (as indication of disorders on the autism spectrum), (4) attention deficit/hyperactivity problems, and (5) oppositional defiant problems. The ASQ-SE assesses social emotional functioning. When comparing the SCT and the control group across the 1–5-year age-range higher incidences of social-emotional functioning problems, affective behavior problems, and pervasive developmental problems became apparent (Urbanus, Swaab, et al., 2020). Risk assessment showed high variability within the SCT group: Some children showed no behavioral problems, whereas others showed behavioral problems at a clinical level. Compared to the control group, children with SCT more often had a clinical or 'at-risk' score for social-emotional problems (40%), affective problems (11%), anxiety problems (16%), and pervasive developmental problems (38%). Further exploring behavioral outcomes in three age groups revealed age-dependent behavioral profiles. In 1-year-old children with SCT, difficulties with social-emotional functioning could already be present, and elevated scores were persistent across the 1–5-year-old age range. Affective and pervasive developmental behaviors were seen in 3-year-olds, and more prominent in 4-5-year-olds. Anxiety, attention deficit, and oppositional defiant behaviors were seen in 4-5-year-olds. Moreover, cross sectional examination of the developmental patterns of affective, pervasive developmental, and oppositional defiant behaviors, showed that risk for neurobehavioral problems increased with age in children with SCT as compared to the control group. Social-emotional problems however, appeared to be more stable and persistent across the entire age range.

Taken together, children with SCT have an increased risk for a range of neurobehavioral problems already at a young age – a risk that appears to increase and expand across behavioral

domains with increasing age. Across the range of behavioral problems, vulnerability in socio-emotional functioning was found to be most prominent, as this showed the highest risk and was found across the full age range.

2. Language and Communication in Young Children with SCT

Language and communication skills were investigated using neuropsychological assessment (i.e., Bayley scales of infant development (Bayley, 2006), clinical evaluation of language fundamentals (CELF) preschool-edition, including pragmatics profile (Korkman et al., 2007; Wiig et al., 2004), Peabody picture vocabulary test (Dunn & Dunn, 1997), MacArthur-Bates communicative development inventories (Fenson et al., 1993), eye-tracking, and arousal (heart rate) measures. These different approaches allowed for a comprehensive overview of various functions within the language and communication domain including both receptive and expressive structural language functions (i.e., phonology, semantics [including vocabulary], syntax), social use of language (i.e., pragmatics), and broader communicative functions (i.e., navigating during social interactions).

Regarding structural language functions, results from our studies showed that compared to an age-matched control group, one-year-old children with SCT produced and understood fewer words and had poorer receptive and expressive semantic skills (Urbanus, Swaab, Tartaglia, Stumpel, et al., 2022). Three- to four-year-old children with SCT in our sample had similar receptive semantic and receptive syntactic language skills compared to children in the control group, but poorer expressive semantic skills. Lastly, five- to six-year-old children with SCT had poorer receptive semantic, expressive semantic, and receptive syntactic language skills. Regarding pragmatic language functions, our results showed that children with SCT between the ages of 3–7-years experienced more difficulties with all three investigated aspects of pragmatic language: Nonverbal communication, conversational routines, and requesting, giving, and responding to information. These difficulties were not only present in children with structural language problems but appeared to be a more common characteristic within the SCT group (Urbanus, Swaab, Tartaglia, Stumpel, et al., 2022). Lastly, when shown videos of communicative interaction, eye tracking measures indicate less

orientation to social aspects in 1-7-year-old children with SCT, in particular to the eyes of the on-screen communicative partner. Physiological measures indicated that children with SCT did not modulate their arousal levels in reaction to different situational demands (i.e., a change in gaze direction) (Urbanus et al, submitted).

To unravel which language and communication functions can serve as building blocks for behavioral outcomes, relations between initial language and communication outcomes (i.e., structural language and pragmatic language) and behavioral outcomes at one-year follow-up were examined. Our results stress the relevance of structural and pragmatic language on later behavioral outcomes. Poorer pragmatic and structural language abilities at baseline were predictive of more attention deficit problems, more pervasive developmental problems, and more social-emotional problems one year later. Poorer pragmatic language at baseline was also predictive of more affective problems and more oppositional defiant problems (Urbanus, Swaab, Tartaglia, Stumpel, et al., 2022).

Taking these results together, language and communication difficulties are present across early developmental stages and various skills within this domain can be affected. Although difficulties with (expressive) structural language functions have been reported previously, this study shows that children with SCT may experience difficulties with communication that extend language abilities. Both comprehension (i.e., receptive abilities) and production (i.e., expressive abilities) can be affected. Language plays an important role in cognitive and social development (Simms, 2007) and is required to communicate one's needs, thoughts, and emotions. Language and communication are also needed for learning, reflecting on experiences, and to understand the world around us. As language and communication are intertwined with many other functions, compromised language and communication abilities could have severe consequences for the development of other neurocognitive functions and behavioral outcomes, consequently also affecting one's ability to participate in society or one's experienced quality of life. In this group of children, pragmatic language in particular was predictive of a broad range of outcomes; social communicative abilities

can serve as an early sign of later behavioral problems and may also help explain the variance in neurobehavioral outcomes in young children with SCT.

3. Social Cognition, Social Behavior and Autism Spectrum Symptoms In Young Children with SCT

Another key area of research was social cognition, social behavior, and autism spectrum symptoms. Our data in 1-7 year old children with SCT reveal vulnerabilities in 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 (Bouw, Swaab, Tartaglia, Jansen, et al., 2022). We also found more social withdrawal during observed social interactions in a structured play situation in children with SCT, aged 1-7 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 (Bouw, Swaab, Tartaglia, et al., 2022).

To explore the extent to which early social vulnerabilities are associated with symptoms that are typical in Autism Spectrum Disorder (ASD), we examined the possible impact of SCT on the early appearance of ASD symptoms. The results demonstrated that ASD symptoms, in particular the domains of social interaction and communication, are substantially higher in children with SCT compared to the general population (Bouw, Swaab, Tartaglia, Wilson, et al., 2022). In our sample, 22% of the children with SCT were at clinical risk for a clinical diagnosis of ASD, including restricted interests and repetitive behaviors. 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 (Bouw, Swaab, Tartaglia, Wilson, et al., 2022).

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. By using eyetracking, we found an impact of SCT on basic social cognitive mechanisms of social orienting to faces and eyes and joint attention, indicating that children with SCT are less inclined to visually orient towards social important 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 (Bouw, Swaab, Tartaglia, et al., 2022). These findings suggest that social behavioral difficulties may be anchored in altered perceiving and processing of social information already early in neurodevelopment.

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. The study showed a significant effect of preventive neurocognitive training on emotion recognition abilities 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 (Bouw, Swaab, & van Rijn, 2022).

Taken together, the results presented in this section concerning the impact of SCT on early social cognitive development, 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 emotions from facial expressions and Theory of Mind. These results suggest a profile of social (cognitive) vulnerabilities in young children with SCT, calling for close evidence-based early

monitoring and targeted support when necessary. Also, our study suggests that there are opportunities for positively supporting development of social cognition, in the domain of facial emotion recognition, in young children with SCT.

4. Emotion regulation, Executive Functioning and Symptoms of Attention Deficit Hyperactivity Disorder (ADHD) in Young Children with SCT

Another area of interest was that of the regulation of emotions, behavior, thoughts, attention, and impulses in order to meet goals and adequately respond to the environment (Blair & Diamond, 2008). Many different interrelated activities are essential to regulation, including having the appropriate emotional response to the situation (not too much nor too little), showing the ability to exert (cognitive) control over your emotions and behaviors, as well as showing an adaptive behavioral response to the situation. The TRIXY Early Childhood Study has showed that young children with SCT are vulnerable in their development of emotion regulation on multiple domains.

The first domain is that of emotional reactivity, the ability to register and respond to emotionally evoking events. We found that when 1-to-7-year-old children with SCT were faced with a stress-inducing event (a robot that emits noise and moves towards the child), they were significantly less aroused than their peers (Kuiper, Swaab, Tartaglia, Cordeiro, et al., 2022). This was measured on a physiological level by assessing heart rate during the event. The results from this study also showed that even when aroused, children with SCT needed a longer period to recover from the event compared to the control group. Interestingly, in another study (Kuiper et al., submitted), these children had a similar arousal and recovery response compared to controls when faced with a more cognitive challenge (e.g., blocked-goal paradigm).

The second domain included behavioral responses during times of stress, in other words whether children with SCT showed different self-regulatory behavior during emotionally evoking events. We found in our studies that used psychophysiological and observational measures that children with SCT have a more limited repertoire of behavioral options than their typically developing

peers. For example, when faced with acute stress, children with SCT showed less facial expressions of emotional distress compared to their peers (Kuiper, Swaab, Tartaglia, Cordeiro, et al., 2022). In addition, the amount of facial expressions was less strongly associated with the physiological arousal response, compared to typically developing children. In other words, the concordance between the physiological reaction and the behavioral response was significantly lower. When it comes to organizing behavior to achieve a blocked goal (e.g., toy out of reach), children with SCT had a more limited range of behavior available to them (Kuiper et al., submitted). In order to get what they want, they showed less constructive (problem-solving) strategies, compared to their peers. Furthermore, children with SCT showed significantly longer use of ineffective strategies with increasing age in this situation, whereas their age-related peers showed a faster decline in use of strategies that were associated with younger age (e.g., venting and avoidance).

The third domain was the development of cognitive skills that are essential to emotion regulation, the executive functions, including inhibition, working memory, and flexibility. The study by Kuiper et al. (2022) revealed that children with SCT are at increased risk for problems with emerging executive functions, from as early as 3 years old, and that those problems appear more pronounced at an older age. Furthermore, impairments in executive functions appear broader than the language domain alone, extending to other areas as well such as flexibility, working memory, and planning. Noteworthy is that executive functioning deficits were increased in the SCT population even when intelligence levels were in the typical 'average' range.

Finally, we examined whether the effect of impaired regulation in behavior already existed from a very early age on. By using a sensitive instrument (the SWAN rating scale) that captures the full range of attentional behaviors that reflect symptoms of ADHD in daily life, it was shown that, on average, the level of ADHD symptoms was higher in the SCT population than in the general population sample, in the full 1-to-6-year age range (Kuiper et al., 2021). The elevated risk was most prominent in the domain of inattention, indicating significant difficulties with regulating attention. There was also a developmental impact: behaviors associated with ADHD increased with age, more

strongly so in the SCT group, although significant differences were observed even in the youngest age-group (1-2-year-olds). Levels of ADHD behaviors were largely similar across karyotypes, although boys with an extra Y chromosome showed more and broader impairments than children with an extra X chromosome. In addition to attention difficulties, boys with 47,XYY also exhibited difficulties with hyperactivity and impulsivity.

Conclusions

The findings of this review suggest that early signs of neurocognitive impairments and symptoms of psychopathology can be identified in young children with SCT. The impact of the additional sex chromosome on neurocognitive development was found on all domains of interest, including language, communication, social cognition, emotion regulation, and executive functioning, albeit in some domains more pronounced than others and with a differential role of maturation (age). This review also provides the initial evidence for early symptoms of neurodevelopmental disorders, including Attention-Deficit/Hyperactivity Disorder (ADHD), autism spectrum disorder (ASD), and communication disorders, across the full range between 1 to 7 year old.

Key to the TRIXY Early Childhood Study was to identify early neurocognitive impairments, as they function as markers for children with an 'at risk' development, that may provide specific targets for early support and intervention. It is important to point out that although differences between children with SCT and controls on average were found with medium to large effect sizes in most of the TRIXY studies, only a subgroup of children with SCT had scores in the clinical range. Thus, while some children with SCT may already be recognized as at risk in their development compared to peers their age, there are also many children with SCT who do not experience any or only mild impairments. Nevertheless, findings from the TRIXY Early Childhood Study showed a "growing into deficit" phenomenon (Rourke, et al., 1983): Neurocognitive difficulties in children with SCT that were present from as early as 1 year old, were found to become pronounced with increasing age. This could reflect increasing problems that may emerge and present more profoundly with age, in relation to increasing demands from the environment. One explanation for this phenomenon is that

neurocognitive functions come 'online' at different and later stages of development, due to the maturation process of the brain, making it possible that the effect of early disturbances on the brain may only become noticeable many years later in development. Important to note here is that most of the studies reported in this review examined the role of age cross-sectionally; longitudinal studies are needed to provide further clarity on the developmental trajectories of children with SCT.

Another key result of the TRIXY study was that neurocognitive skills may present as very relevant candidates to serve as specific targets for early support and (preventive) intervention in children with SCT. Not only did the results show a predictive value of neurocognitive impairments (in the domain of language and communication) with regards to later psychopathology, our results also provide the initial support for the effectiveness of neurocognitive training (in the domain of social cognition). These promising results suggest that there are opportunities for positively supporting the development of social cognition in children with an extra X or Y chromosome.

The study of genetic conditions, such as sex chromosomal trisomies, comes with specific recruitment and study design challenges (Prasad & James, 2009). Specific to SCT is the low diagnosis-rate whilst the prevalence of the condition is relatively high, making it difficult to include enough participants that cover the full range of potential phenotypic expression and not only those who experience difficulties in daily life that led to diagnosis of the chromosomal aneuploidy in the first place. The approach that was used in the TRIXY Early Childhood study was to record how families came to learn of the study to correct for potential recruitment bias and three subgroups were identified: a) 'active prospective follow-up' (largest group in the full cohort), b) 'information seeking parents', and c) 'clinically referred cases' (smallest group in the full cohort). The studies reported in this review controlled for recruitment condition in their analysis and found no significant influence of recruitment bias: how parents enrolled in the study did not affect the study results. This may suggest that a) that the children participating in the TRIXY Early Childhood Study reflect a representative subsample of diagnosed children with SCT, and b) that clinical examination of the developmental impact is highly relevant in children with SCT. Having a (prenatal) diagnosis of SCT does not reliably

predict what the exact outcome will be for any given individual, highlighting the importance of close monitoring throughout development and to act promptly with guidance and tailored-made (preventive) interventions when needed.

When it comes to the specific karyotypes, 47,XXX, 47,XXY, and 47,XYY, most of the studies included in this review found no significant differences between the specific karyotypes on neurocognitive and behavioral outcomes. The overall absence of significant differences suggests that children with 47,XXX, 47,XXY, and 47,XYY karyotypes may show substantial similarities in their neurocognitive and behavioral outcomes, which fits with findings in neuroimaging studies showing convergent effect of the X and Y chromosomes on brain structure (Raznahan et al., 2016). Nonetheless, boys with an extra Y chromosome in our sample at some points also showed a slightly more impaired profile. It should be mentioned however that the group of boys with 47,XYY was relatively small compared to the other two karyotype groups and results that included the comparison of children with 47,XYY and the other groups should be interpreted with caution. Overall, these outcomes suggest that the observed vulnerabilities may represent rather general 'stable' vulnerabilities associated with the genetic condition, than karyotype-specific vulnerabilities.

Within the TRIXY Early Childhood study, some of the children were treated with testosterone supplements. Whereas early testosterone treatment is considered evidence-based practice when treating a micro-penis in young infants with Klinefelter's syndrome, far less is known about the impact of early testosterone treatment on neurocognitive development. In order to address this, Aksglaede and colleagues (2020) recommend to wait for larger, randomized and placebo-controlled studies to investigate the potential beneficial side effects of testosterone in children with Klinefelter syndrome. Therefore, within the TRIXY Early Childhood Study we did not analyze outcomes stratified by testosterone treatment. Preliminary results from studies examining the effects of early testosterone treatment in SCT (Davis, 2022, this issue) showed no significant differences on any neurodevelopmental outcomes measured. Future studies are needed to provide evidence-based recommendations to guide clinical care in infants with Klinefelter syndrome.

The TRIXY Early Childhood Study was made possible by the collaboration and extensive effort of multiple international research sites. It is thanks to all medical centers, researchers, and patient organizations involved, that over a 100 families with young children with an additional X and Y chromosome were part of the study. It is imperative for empirical studies that examine genetic conditions to include a large enough sample to capture the wide heterogeneity in cognitive and behavioral outcomes so often described in these genetic populations. Thus, we strongly encourage more and closer international collaboration in order to advance the overall research and clinical field regarding sex chromosomal aneuploidies. This is also supported by our data, given that almost all of the reported studies in this review did not find significant differences between recruitment sites (USA vs. the Netherlands).

When it comes to clinical care, the results of the TRIXY Early Childhood Study can have important clinical implications. Working in a clinical setting with children with SCT, professionals need to be aware of the variation in (neurocognitive) functioning between children with SCT just as much as the developmental risk for impaired (neurocognitive) functioning. From a young age, difficulties with neurocognitive functions (including language, emotion-regulation, executive functioning, social cognition) can be part of an individual's cognitive profile, even in the face of average intelligence. Identifying risk for impairments in (specific areas of) neurocognitive functions can result in specific guidelines on what function needs to be supported during treatment. In addition, while these results indicate that neurocognitive training might be a valuable component in treating difficulties in children with SCT, it is crucial not to focus narrowly on these specific neurocognitive functions alone, but also address the social, emotional, and behavioral development in relation to the social context in which a child with SCT grows up, such as family and school.

The research field of SCT is in need of more longitudinal designs, in order to study the developmental pathways of individuals with SCT from early childhood into school-age, adolescence, and adulthood. In addition, future studies should investigate the role of environmental factors on the variability in outcomes in SCT, including family and parental factors, which may also pinpoint

protective factors. In terms of advancing the clinical care for these individuals, examining which interventions are effective in minimizing the developmental impact of SCT would be an important area of future research as well.

The main conclusion to be drawn is that (a subset of) children with an extra X or Y chromosome are vulnerable from a very early age on, on numerous important neurodevelopmental domains, including language/communication, social cognition, and emotional- and behavioral regulation. Increased symptoms of neurodevelopmental disorders are found in young children with SCT compared to typically developing peers. Our findings from the TRIXY Early Childhood Study are among the first to show that individual differences between children with SCT already exist in early childhood and that these can be predictive of future psychopathology, including behavioral symptoms. It provides support that neurocognitive functions work as underlying building blocks for future development and that these at-risk markers can also be targeted for preventive interventions. In addition, these collaborative results demonstrate that genetic populations that can be identified early in life (even before birth) can serve as a natural at-risk model to examine early pathways into psychopathology.

CHAPTER 7

Summary and general discussion

Summary and General Discussion

One in every 650 to 1000 children has a genetic condition known as a sex chromosome trisomy (SCT), indicating that they are born with three sex chromosomes instead of the typical number of two (Berglund et al., 2019). Karyotypes that results from SCT are 47,XXX (also known as Trisomy X), 47,XXY (also known as Klinefelter's syndrome), and 47,XYY. Individuals with SCT are at increased risk for psychopathology, referring to social, emotional, and behavioral problems (Geschwind et al., 2000; Ross et al., 2012; Tartaglia et al., 2010; van Rijn et al., 2008). By considering behavior on a continuum ranging from adaptive to non-adaptive, having significant behavioral problems can tremendously impact adaptive day-to-day functioning to such an extent that these behaviors can also be classified as symptoms of psychiatric classifications (according to the DSM-5, APA, 2013). For individuals with SCT, an increased risk for psychiatric classifications has been described (van Rijn, 2019), including affective disorders, social-communicative disorders, and neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). Around 25% of the SCT population is at clinical risk and thus experience a significant impact of the additional X or Y chromosome on their mental well-being and day-to-day functioning.

To understand how the extra sex chromosome impacts development and contributes to daily life outcomes, a neurocognitive perspective can provide insight in how differences in information processing contribute to psychopathology. In other words, how neurocognitive functioning may serve as an underlying and possible driving mechanism between genes, brain, and behavior. Thus far, studies have shown that a significant fraction of genes on the X chromosome are associated with brain development and functioning (Zechner et al., 2001). Furthermore, structural and functional differences in the brain have been identified in individuals with SCT compared to the general population (Steinman et al., 2009; Warling et al., 2020). In addition, impairments in neurocognitive functioning in SCT are described in adolescence and adulthood. These include differences compared to the general population in the area of intelligence, language, executive functioning, and social

cognition (for a review, see van Rijn, 2019). Studies on neurocognition in early childhood, before the age of 8 years old, are scarcely available (Urbanus, van Rijn, et al., 2020). Because most neurocognitive functions start to develop in early childhood, a developmental approach is of key importance here. Deviations from typical neurocognitive development could pinpoint at-risk markers in development in children with SCT and potentially indicate a suboptimal process of brain maturation (Urbanus, van Rijn, et al., 2020). Within this dissertation, the goal was to identify early developmental risks in young children with SCT, with a specific focus on neurocognitive skills essential to adequate social and emotional adaptation, namely self-regulation. The regulation of thoughts, emotions, and behavior in a social context is a vital factor when it comes to daily life functioning and therefore an interesting neurocognitive candidate to study in relation to psychopathology. This knowledge would increase our understanding of neurodevelopmental pathways into later psychopathology and uncover specific targets for early and preventive intervention.

The study of early development in children with SCT becomes more relevant every day: With the increasing use of non invasive prenatal testing worldwide, the number of children who are prenatally diagnosed with SCT are expected to increase (Samango-Sprouse et al., 2017). This has two important implications. First, it highlights the urgent need for more updated knowledge on the development of children with SCT in order to guide clinical care. This knowledge would inform clinicians and parents on the potential developmental impact of SCT and help develop neurocognitive intervention tools. Second, it allows for the unique opportunity to prospectively study these children before any severe developmental problems or delays unfold and provide input for a high-risk model to examine early neurocognitive factors in relation to later psychopathology.

Whilst the somatic profile of individuals with SCT has received increasing scientific interest over the last decade (Pieters et al., 2011), the behavior and neurocognitive development of young children with SCT remains poorly understood (Urbanus, van Rijn, et al., 2020). To enhance our understanding of early development of children with SCT and how differences in behavior and

neurocognitive functioning contributes to later psychopathology, the TRIXY Early Childhood Study was initiated. Through means of extensive international and national cooperation, the TRIXY Early Childhood Study was able to include a uniquely large sample of young children with SCT to capture a broad range of developmental outcomes in this population and compare them with age-matched sample of typically developing children. The general aim of the TRIXY Early Childhood Study is to examine behavioral and neurocognitive mechanisms that are not only known to be important in typical childhood development but also identified areas of vulnerability in individuals with SCT. Unraveling differences in neurocognitive functioning and behavior would help us understand the different pathways into later psychopathology, but would also identify vulnerable areas of development suitable for early and/or preventive intervention.

The studies in this dissertation specifically focused on one area of vulnerability: self-regulation. Self-regulation refers to an interrelated set of skills needed to regulate our emotions, thoughts, impulses, and behavior. Several aspects of self-regulation develop at an accelerated pace in early childhood (Blair & Diamond, 2008), partly due to brain maturation (Posner & Rothbart, 2000), highlighting both its usefulness and relevance when studying at-risk neurocognitive pathways in the young developing child. Moreover, self-regulation has been identified as an important transdiagnostic factor in the development of psychopathology in the general population (Romer et al., 2021) and has significant relevance to daily functioning and quality of life (Blair & Diamond, 2008). Being able to regulate your emotions, thoughts, and behavior promotes positive adjustment and adaptation in daily (social) life, with the latter often being a challenge for individuals with SCT, who are typically described as having emotional, behavioral, and social difficulties in adolescence and adulthood. The central aim of this dissertation was to provide a better neurocognitive understanding of early self-regulatory skills in young children with SCT by using a comprehensive approach. Sensitive and direct measures of self-regulation were used and included not only measures of what can be observed and tested directly in terms of behavior and neurocognition but also included measures of more fundamental processes such as physiological responses, that are able to represent

the arousal component of emotion regulation. These measures collectively have the ability to identify how (deviations in) information processing influence and shape behavioral responses in order to function adequately.

This dissertation focused on how children with SCT regulate their emotions, thoughts, and behavior, as expressed in behavioral regulation (symptoms of ADHD), cognitive regulation (executive functions), and emotion regulation (arousal, expression, and coping behavior) in a sensitive period of development. The total sample described in this dissertation consists of 107 children with SCT, between the ages of 12 months and 7 years old, that were compared to 102 age-matched typically developing peers. Children with SCT were recruited with the help of clinical genetic departments, pediatricians, and national advocacy or support groups, in the Netherlands as well as in Denver, USA. The SCT group consisted of 33 girls with 47,XXX, 50 boys with 47,XXY, and 24 boys with 47,XYY. As part of the longitudinal design of the study, children were assessed either at home or at a University lab during an initial baseline assessment and a follow-up assessment 12 months later (with a subgroup also having a 1-month post-intervention assessment). The experimental studies described in this dissertation only include data collected at baseline. During assessment, several aspects of self-regulation were measured. At the behavioral level, parent-reported data on attention and behavioral problems (ADHD-symptoms) was assessed, along with observational data of emotion regulation strategies and emotional expression. At the level of neurocognitive functioning, both test performances on executive function tasks as well as parental report of executive functioning in daily life was assessed. At the level of psychophysiology, arousal during emotion-evoking situations was assessed using heart rate data as an indicator of emotional reactivity. To examine the developmental impact, age was a significant factor of interest in all studies. This focus on early childhood was crucial to better understand the developmental impact of SCT in young children and for pinpointing targets to serve as markers for preventive and early intervention.

Taken together, the results in this dissertation reveal that children with SCT are a significant risk for self-regulatory difficulties, that appear to be more pronounced with increasing age. Early signs of self-regulation problems were found at a behavioral level, expressed in more symptoms of inattentive behavior (as seen in ADHD-symptomatology) in children with SCT (**Chapter 2**). Although these problems were present as early as 12 months old, older children with SCT show more pronounced inattentive behavior. To adequately regulate behavior, thoughts, and emotions, executive functions are essential neurocognitive skills. However, children with SCT have less strongly developed executive functions to rely on, even with average intelligence levels (**Chapter 3**). Again, older children with SCT show more pronounced but also broader problems in the area of executive functioning. Part of adequate self-regulation are also those skills involved in emotion perception and the regulation of emotions. On a physiological level, children with SCT show a more blunted arousal response (in terms of heart rate) to a stress-evoking event and need more time to recover from this event, compared to their peers (**Chapter 4**). Also, they display significant less expression of negative emotions during stress than their peers. Moreover, their level of arousal was less predictive of a congruent emotional expression: The overlap between arousal and expression was significantly lower in children with SCT. Furthermore, when confronted with a frustration task, children with SCT show a more limited range of behavioral coping strategies and, with increasing age, tended to rely longer on emotionally “immature” regulation strategies (**Chapter 5**). Together, these studies indicate a significant impact of SCT on early development in terms of self-regulation. However, self-regulation is not the only important neurocognitive building block in early childhood that appears vulnerable in SCT. The review (**Chapter 6**) showed that in addition to differences in self-regulation, children with SCT are vulnerable on numerous important neurodevelopmental domains, including language, communication, and social cognition.

Below, the results of the five studies are summarized in more detail, followed by a general discussion of the main conclusions, implications, directions for future research, and a summary of the main findings.

Main Findings

Attention and Behavioral Regulation (ADHD-Symptoms)

The study in **chapter 2** addressed to what extent young children with SCT are able to regulate their actions in daily life, by examining behavioral psychiatric symptoms. The most salient markers of impaired self-regulation to study in early childhood are those behavioral symptoms associated with Attention-Deficit Hyperactivity Disorder (ADHD): a neurodevelopment disorder characterized by severe symptoms of inattentiveness, hyperactivity, and impulsivity that interfere with daily functioning and development (DSM-5: APA, 2013). Rather than considering ADHD as an all-or-none phenomenon (e.g., classify these children as meeting diagnostic criteria for ADHD or not), the present study examined variation in ADHD symptoms as a key factor of interest to provide a more sensitive measure of early regulation deficiencies in young children. We used a sensitive, well-known, and widely used instrument (the SWAN rating scale) that was able to capture the full gradient of attentional behaviors reflected by symptoms of ADHD in daily life as reported by their parents. The results of this study showed that, on average, the level of ADHD symptoms in SCT was higher than in the general population sample in the full 1-to-6-year age range (with medium effect size). More specifically, children with SCT had more behavioral challenges in the domain of inattention reported by their parents (with large effect size), indicating significant difficulties with regulating their attention. Differences with control peers were already evident for the youngest age-group (1-2-year-olds), but also present in the 3-4-year-olds and 5-to-6 year-olds. Furthermore, inattentive ADHD symptoms increased with age for children with SCT, whilst for controls ADHD symptoms were not related to age and appeared to present relatively similar across ages. From a clinical perspective, 24% of the children with SCT had scores in the clinical range on parent-report, indicating the severity of ADHD symptoms and the increased risk for ADHD symptomatology in the SCT population. Levels of ADHD symptoms were largely similar across karyotypes, although boys with an extra Y chromosome

showed more and broader impairments than children with an extra X chromosome. In addition to inattention difficulties, boys with 47,XYY also exhibited hyperactivity and impulsivity. Ascertainment bias (how children enrolled in the study) and country of recruitment (whether children were tested in the Netherlands or in the United States of America) were not relevant to the increased risk of ADHD symptoms, underlining the robustness of these findings.

These results suggest that self-regulatory difficulties already exist in very young children with SCT, pointing to a significant neurodevelopmental risk from toddlerhood onward. Given the fact that a significant fraction of the genes on the sex chromosomes are involved in brain development, this elevated risk for behavioral difficulties may be one of the first signs that the child's genetic makeup has impacted the brain's development and, more specifically, the brain areas that are important for self-regulation. This elevated risk for self-regulation in terms of ADHD-symptoms we found in young children corresponds to what has been found in older children, adolescents, and adults with SCT (van Rijn, 2019): in these samples, estimates of significantly elevated clinical levels of ADHD symptoms fall around 35% for 47,XXY (with a range of 27-42%); 49% for 47,XXX (with a range of 27 52%); and 69% for 47,XYY (with a range of 62-76%), with inattentive symptoms being most common.

Cognitive Regulation (Executive Functions)

The study reported in **Chapter 3** focused on the development of neurocognitive skills essential to self-regulation: executive functions. In early childhood, children start to develop the ability to act purposefully and goal-directed, which is supported by the emerging development of executive functions during this period. Executive functions are interrelated neurocognitive skills essential to learn, cope, and manage daily life (Diamond, 2013) and thus of vital importance to support adequate self-regulation (e.g., to regulate your emotions, thoughts, and behavior). Several executive functions are attention, inhibition, monitoring, flexibility, working memory, planning, and fluency (Anderson, 2001).

To investigate the development of emerging executive functions in children with SCT, we compared the performance on several executive function tasks and parental report on daily life executive function behavior of children with SCT to that of a population based sample. Results showed that impairments in executive functions were significantly more prevalent in SCT than in controls and already present from the age of 3 years old. Specific impairments were found in the domain of verbal executive functions and working memory (with medium effect sizes), in addition to broader and more pronounced impairments in older children with SCT. Children with SCT aged 5 to 7 years showed difficulties with global executive functions, verbal fluency, cognitive flexibility, emotional control, working memory, and planning and organizing (with medium to large effect sizes). In addition to general group effects, the results of the study also showed that for some executive functions, there was an interaction (group by age) effect. Whereas older children in the control group showed improved planning and organizing behaviors with increasing age, children with SCT continued to struggle in this domain and did not improve with increasing age. In addition, we found that impairments in executive functions were present across the broad range of intelligence levels. Thus, difficulties with executive functions can be part of the neurocognitive profile of children with SCT, and was not only limited to those children with a below average intelligence.

Although impairments in executive functions have been described in school-aged children, adolescents, and adults with SCT (Janusz et al., 2020; Lee et al., 2015; Ross et al., 2008, 2009; Samango-Sprouse et al., 2018; van Rijn & Swaab, 2015), this study is the first to show that there is a developmental impact of SCT on emerging executive functions before the age of 7 years and that children with SCT are at significant risk for difficulties with executive functions already in early childhood. This increased risk in children with SCT indicates that their ability to show purposeful, goal-directed, and problem-solving behavior can be affected, from as early as 3 years old. The impact for these children is significant, given that preschool executive functions are vital for school readiness (Blair & Razza, 2007), academic success (Gathercole et al., 2004), and psychological well-being in general (Kusche et al., 1993). Our results suggest that emerging executive functions could be one of

the key components in explaining the variability as well as the increased risk for psychopathology in this genetically at-risk group. This is further supported by evidence from other studies in individuals with SCT that have linked impairments in executive functions with social-emotional and behavioral problems (Skakkebæk et al., 2017), psychotic symptoms (van Rijn et al., 2009), and neurodevelopmental disorders, such as ADHD symptoms (Lee et al., 2011) and ASD symptoms (van Rijn et al., 2012).

Emotion Regulation (Physiological Arousal, Expressivity, and Coping Strategies)

In addition to executive function skills that help to regulate behavior, children also need to process emotional information and regulate their emotions. The manifestation of emotions and its regulation includes multiple biological, cognitive, and behavioral systems, which can be assessed by examining physiological changes and behavioral responses as indicators of these systems (Tracy, 2014). In studies described in **Chapters 4 and 5** examine the interplay between the reactivity of the autonomous nervous system (physiological changes in terms of arousal) and behavioral responses in children with SCT, during different emotion-evoking situations.

The study reported in **Chapter 4** examined how children with SCT process emotions during a stress-evoking event (e.g., toy that unexpectedly approaches the child and makes threatening sounds), by collecting both physiological (heart rate) and observational data (expression of negative emotion). By studying both parameters with sensitive and objective techniques, the study aimed to identify differences in emotional processing compared to controls. Firstly, the study identified different reactivity and recovery patterns for children with SCT. More specifically, children with SCT showed a significantly lower arousal response (in terms of heart rate) following a stress-evoking event. This indicates that children with SCT show a more blunted response in response to a stressful event. Furthermore, the results showed that even when the stressful event had ended, the recovery of the physiological system to its original baseline state took longer in children with SCT compared to controls. To compare, typically developing children showed an immediate physiological response and

were able to recover quickly to their original baseline state when the event had ended. In addition to these differences in emotional arousal, we found that children with SCT showed less expression of emotion in their face or body during the stressful event. Furthermore, there was less overlap between the physiological and behavioral components of the emotion response. Children with SCT showed a significantly lower concordance between emotional arousal and expressivity compared to controls.

In addition to how children with SCT experience emotionally evoking events, the study reported in **Chapter 5** examined what young children with SCT do in terms of the regulation of emotions: how do they shape their behavior when faced with challenges? To what degree are these children able to adjust their behavior accordingly so that they can acquire and accomplish their goals, despite arising emotions? We investigated what the behavioral strategies are of children with SCT during a frustration-evoking event (e.g., locked-box with desirable toy). The results show that whilst children with SCT are similarly frustrated by the situation as their peers (displayed by similar physiological changes), they show a diminished behavioral response to the event. Structured behavioral observations revealed significant differences between groups in the use of constructive emotion regulation strategies: children with SCT show less problem-solving strategies in the face of frustration than their typically developing peers. An interesting developmental effect was also found; whereas the use of avoidance strategies tends to decline with age in typically developing children, this was significantly less so in children with SCT. Thus, these results suggest that children with SCT may have more difficulties regulating their emotions as compared to typically developing children, may have a more limited range of behavioral strategies to implement, and tend to rely longer on more inefficient and emotionally “immature” emotion regulation strategies with increasing age.

The results of the studies reported in **Chapter 4 and 5** show that differences in emotion processing and emotion regulation are already part of the early developmental profile of young children with SCT. To date, no other studies have examined these self-regulation processes this early

in the life of children with SCT. By combining novel, state-of-the-art, and sensitive techniques we were able to provide insights in the fundamental mechanisms of how young children with SCT perceive, process, and regulate emotions. This is especially relevant, given that these processes are traditionally challenging to study in young children (Bölte et al., 2016), for example with more traditional pen-to-paper tasks that require cognitive and language responses: skills that are yet developing in early childhood.

The results of the current studies are of additive value to the literature on emotion regulation in individuals with SCT. Most studies have focused on behavioral measures and questionnaire data and have shown that ineffective emotion regulation strategies are typical to adolescents and adults with SCT and include behaviors such as emotional outbursts, depressive symptoms, and increased anxiety (Tartaglia et al., 2010; van Rijn & Swaab, 2015). Our results also match with other studies that found deviations in emotional expressivity, including difficulties in expressing negative emotions to others (van Rijn et al., 2008), regulating their emotions (van Rijn & Swaab, 2020), and identifying and verbalizing their emotions (Van Rijn et al., 2006). However, only three other studies to date focused on the physiological indices of emotional reactivity similar to our studies, with discordant results. To illustrate, one showed increased affective arousal in response to viewing emotion-evoking visual images (van Rijn, Barendse, et al., 2014), one showed similar arousal responses to sensory stimuli, and the third found a blunted affective arousal response to evoking social stimuli in young children with SCT (Urbanus et al., n.d.). Differences in these findings might relate to the nature of the stressor (e.g., social, non-social, neutral) and the context in which the arousal is measured (Stifter et al., 1989). These inconclusive results highlight the importance of further investigation of emotional processing in individuals with SCT.

Neurocognitive Building Blocks in Early Childhood (TRIXY Early Childhood Study)

To fully understand the early developmental profile of children with SCT, it is also important to examine their development in a broader context, rather to focus on one domain specifically. Many

other factors are important to childhood development, including language, communication, social cognition, and symptoms of ASD. The review in **Chapter 6** aims to provide an overview of this and includes a description of the collective results from the TRIXY Early Childhood study. Whereas the experimental studies described in this dissertation show that several aspects of self-regulation are impacted in children with SCT, the results described in the review show that it is not a singular vulnerable domain of development. The results of the collective TRIXY Early Childhood study show that in addition to self-regulatory problems, impairments in social cognition, language, and communication are also part of the behavioral and neurocognitive profile of young children with SCT. Interestingly, in this review we also addressed the initial evidence and potential use of identifying neurocognitive problems, such as social cognition, to implement an early preventive intervention to positively support development in this population. These collective results highlight the importance of a comprehensive and inclusive approach when studying genetic conditions such as SCT.

Conclusions

In sum, the studies included in this dissertation show that children with SCT are vulnerable in their ability to self-regulate. By implementing a neurocognitive approach to the study of the development of children with SCT by using sensitive, state-of-the-art methods and measures, we found that having an extra X or Y chromosome impacts the development of these children on multiple levels of functioning. Not only did we find impaired psychophysiological responses of self-regulation, including a blunted arousal response, we also found individual differences in cognitive skills important for self-regulation: executive functions. Furthermore, the effect of impaired self-regulation was found on the emotional and behavioral level of functioning and included differences in the ability to regulate attention (as reflected in high levels of ADHD-symptoms) as well as diminished emotional expressivity and inefficient use of regulation strategies. Furthermore, in most of the studies, a developmental impact of SCT was found: differences in functioning with typically developing peers were present as early as at 12 months old and increasing and more pronounced

differences with typical developing peers were found with increasing age. Despite variation in karyotype (XXX, XXY, and XYY), recruitment strategy (i.e., the reason for enrollment in the study), and timing of diagnosis (prenatal or postnatal), differences between the SCT and control group remained present indicating that we found a robust at-risk neurocognitive profile in this population.

Combined, these studies show clear indications that the self-regulation abilities of children with SCT can be impacted, already from an early age. Because the ability to self-regulate is of significant relevance to daily life functioning and adaptation (Blair & Diamond, 2008), we found substantial evidence in our research studies that self-regulation (expressed in ADHD-symptoms, executive function problems, and emotion regulation problems) is a vulnerable domain in individuals with SCT. Thus, self-regulation can be considered a key underlying mechanism that can contribute to the increased risk for psychopathology in SCT. To illustrate how early self-regulatory skills may contribute to later social, emotional, and behavioral problems, we provide an overview of our lessons learned and how they interact as well as influence the development in young children with SCT.

First, the blunted physiological arousal response we found (**Chapter 4**) indicates that children with SCT are impacted in their ability to rely on their innate system to signal situations as emotionally relevant. As a result, children can miss relevant situations and thus opportunities to interact with their environment (Gross, 2013). This is imperative in early childhood, because being able to interact with your environment is essential to learn that emotion-evoking events can induce emotional stress, but also that this stress can be regulated effectively and successfully (Beeghly & Tronick, 2011). Second, on a neurocognitive level, we found that the executive functions are vulnerable in children with SCT (**Chapter 3**), indicating that these children can be more limited in guiding their thoughts, emotions, and behaviors towards an adaptive response. In other words, they experience more difficulty in organizing their behavior in such a way that it enables them to make adaptive choices in accordance with their goals and to also consider the feelings and actions of others. This has a

significant impact for future development, especially given that executive functioning in young children is an essential precursor for the development of social skills (Hughes & Leekam, 2004). Thus, children with SCT might be dually impacted: not only might they experience more difficulty in relying on their internal compass (e.g., emotional reactivity), their cognitive skills to adequately shape emotional experiences, thoughts, and behavior may also be less developed as well. Three, as a potential result of these impaired processes, children with SCT might have more difficulty shaping adaptive behavioral responses (e.g., experiencing emotional outbursts, depressive symptoms, and social-communication problems), as is reflected in a diminished use of emotion regulation strategies (**Chapter 5**), diminished emotional expressions (**Chapter 4**), and increased symptoms of psychopathology, including ADHD (**Chapter 2**).

The fact that these studies found self-regulation difficulties in addition to other neurocognitive skills such as language, communication, and social cognition as early as 12 months old (**Chapter 6**) indicates that the impact of the X and Y chromosome on development is likely associated with a suboptimal brain development and broad maturation processes in individuals with SCT. This is not surprising, given that a significant fraction of genes on the X chromosome are associated with brain development and functioning (Zechner et al., 2001). The vulnerabilities in the neurocognitive domain of young children with SCT will undoubtedly have a significant impact on their day-to-day-functioning and future development and even more so, should these vulnerabilities co-occur. Hypothetically, children with SCT who are impaired in their ability to interpret emotional expressions in other people's faces (e.g., the skill to recognize emotions, part of social cognition), might experience more difficulty in interpreting these expressions and understand social cues and interactions with others. This would be even more challenging when their ability to focus their attention on important cues is impacted as well. Combining this with limited options to shape your behavior in a goal-directed manner including considerations about the feelings and actions of others, our social world probably is experienced like a complex and challenging environment to grow-up in.

Clinical Implications

Not only did these studies enhance our knowledge of early development in young children with SCT, they also identified at-risk markers in development on multiple levels of functioning which in turn help shape tailored-made interventions. Specific to the self-regulation results presented in this dissertation, we learned that 1) children with SCT are less able to rely on their internal, emotional compass, 2) that this internal response is less predictive of a behavioral response (display of emotions or coping strategies), 3) children with SCT show more difficulty in cognitively regulating of their emotions, thoughts, and behavior (top-down regulation), and 4) children with SCT tend to rely longer on inefficient, emotionally immature behavior regulation strategies in emotionally-evoking situations. Awareness of these self-regulatory skills and the difficulties children with SCT might experience may be meaningful in when providing psychoeducation and intervention strategies. Parents and clinicians may need to 1) help verbalize and address emotionally relevant situations to the child (i.e., signal situation as relevant by functioning as an external compass), 2) understand that the display of emotions of behavior might not be the same as the internal experience of the emotion of the child, 3) help improve the cognitive skills, including executive functions and language, to help shape an adaptive response in a cognitive manner as well as 4) help shape and teach alternative behavioral strategies in order to cope with emotionally challenging situations. The results of these studies illustrate the usefulness of a neurocognitive perspective in SCT, both empirically and clinically.

The knowledge provided in this thesis is essential to improve (prenatal) genetic counseling as well as clinical care in terms of diagnostics and treatment. Since increases in diagnosis rate is to be expected due to increasing access to NIPT worldwide, an increasing number of parents learn of the genetic condition of their child during prenatal testing (Samango-Sprouse et al., 2017). Our results

contribute to the growing knowledge on the development of children with SCT, essential to adequately inform parents and health care professionals on the full gradient of potential outcomes associated with having an extra X or Y chromosome. For children that are diagnosed, the current results highlight that integrating a developmental perspective in diagnostics and treatment is key. Even though these results show that children with SCT show certain neurocognitive difficulties at certain periods in time *on a group level*, the cognitive profile of an individual child with SCT can still largely vary, also from time to time. Thus, having a genetic condition per se does not reliably predict the future outcomes and perspective for an individual child. Even more, our studies indicate that skills may appear age-appropriate at one age in development, they may potentially grow into a greater delay later in development, the so called "growing into deficit"-phenomenon, (Rourke et al., 1983)). Therefore, (repeated) individual clinical neuropsychological assessment of important neurocognitive skills (including self-regulation, executive functioning, language, communication, social cognition) is highly advised. Children with SCT should preferably be seen at crucial moments in development (developmental milestones) and/or when problems arise. As a clinician, being aware of the variability and increased risk in neurocognitive functioning, even when intelligence levels are in average range, could increase the use of evidence-based interventions that are tailored-made to individual impairments as well as their strengths. Ideally, these interventions would also consider the somatic, psychological, and environmental (family and school) factors as well. Therefore, close collaboration with other disciplines involved in the clinical care for individuals with SCT, such as neurologists, endocrinologists, physical therapists, occupational therapists, pediatricians, language or speech therapists, and (neuro)psychologists, is highly encouraged (Tartaglia et al., 2015). Both in the Netherlands and in the state of Colorado in the United States of America, such a collaboration has been formalized in centers of expertise for individuals with a trisomy of the X and Y chromosomes (TRIXY Center of Expertise and eXtraordinarY Kids Program). In these centers, different professionals with expertise on SCT join together to facilitate and improve health care of individuals with SCT.

Future Directions

In future work, investigating the developmental pathways from early childhood into school-age, adolescence, and adulthood is important to further determine the predictive value of neurocognitive skills as well as its effectiveness as targets for intervention. It will be imperative that future research incorporates a longitudinal design to validate the age-dependent effects found cross-sectionally in this thesis. When it comes to interventions, we know of only one neurocognitive intervention (pilot) that has been systematically examined in young children with SCT (Bouw, Swaab, & van Rijn, 2022), with promising results showing that an intensive parent-child training of emotion recognition skills can increase social cognitive skills in children with SCT. Future research should further explore whether early (and/or preventive) neurocognitive training could minimize the developmental impact of SCT on daily life outcomes. Elaborating on this hypothesis, future research should look for existing interventions that focus on training a specific neurocognitive skill (such as "Transporters" used in (Bouw, Swaab, & van Rijn, 2022)) as well a designing SCT-specific interventions (such as a social self-management training (Martin et al., 2020)).

Future studies should also examine the underlying dynamics between the several neurocognitive vulnerabilities found in children with SCT. For example whether a cumulative risk is at play (e.g., additive effect of risk factors) or whether the interplay between specific factors is more important. A cumulative risk approach would offer a method for investigating how risk factors operate in the context of one another to influence child outcomes (Appleyard et al., 2005) and could thus provide important guidelines on the timing of early and preventive interventions.

Finally, to enhance our understanding of potential outcomes in order to improve clinical care, the study of protective factors and strengths of both the child and its environment would be essential too. The research presented in this dissertation shows that the area of emotional control and regulation is a distinct area of vulnerability in children with SCT. However, when it comes to

emotion regulation, many familiar factors are known to influence the development of emotion-regulation skills of typically developing children (A. S. Morris et al., 2007). Amongst those are parenting skills and behaviors related to emotions. Through their parenting skills, parents react to the emotions of the child that can influence the emotion regulation skills of the child (Eisenberg et al., 1998). These reactions can be positive (e.g., validating, rewarding) as well as negative (e.g., punishing, denying or down-playing emotions). In addition, parental characteristics such as their own emotion regulation skills, mental well-being, and familial (psychiatric) history, are viewed to be important factors in the development of childhood emotion-regulation as well (A. S. Morris et al., 2007). Interesting, emotion regulation skills of the child has been identified as a mediator between parenting behavior and externalizing behavior in typically developing children (Eisenberg, Gershoff, et al., 2001). It would be interesting to examine how parenting skills and familial factors interplay with the neurocognitive profiles and other characteristics of children with SCT in future studies, both directionally as well as bi-directionally.

Summary of the Main Findings

In this thesis, various neurocognitive and behavioral components involved in self-regulation were explored in a large international cohort of young children with SCT during a critical period of development (1 to 7 year old). Following the neurocognitive perspective that information processing deficits can contribute to psychopathology, this dissertation focused on three important interrelated elements of self-regulation: behavioral regulation, cognitive regulation (in terms of executive functioning), and emotion regulation.

- By combining sensitive and innovative techniques, such as reactivity of the autonomous nervous system, with structured observations and cognitive tests, specific vulnerabilities in different levels of self-regulation (as expressed in behavioral, cognitive, and emotional responses) can be identified in children with SCT. These findings are essential to guide clinical care in terms of early assessment and treatment, but also vital to improve genetical counseling on the developmental profile of these children.

- On the behavioral level, children with SCT show more symptoms of ADHD such as inattentiveness in daily life compared to typically developing peers. Albeit not all children with SCT meet full diagnostic criteria for ADHD, many children will present ADHD-like behaviors throughout early childhood. On a cognitive level, children with SCT show impairments in their ability to regulate their thoughts and behaviors, in terms of executive functioning. Specific vulnerabilities were found in the area of working memory, cognitive flexibility, verbal fluency, and planning. Other areas of executive functions appeared to be intact, such as inhibition and self-monitoring. On the emotional level, it was found that several processes involved with emotions, including reactivity, responsivity, expressivity, and regulation, appears different in children with SCT. When faced with stress, children with SCT have a less sensitive arousal system (expressed in a blunted physiological response), show less emotional expression than can be expected based on age, and show a more limited range of regulatory behaviors that would otherwise be helpful in coping with stressful situations.
- Emotional responses in children with SCT that can be directly observed in behavior (e.g., facial or bodily expressions) may not necessarily reflect a child's level of arousal to these emotions. The connection between the external display (emotions) and the internal arousal response (in terms of heart rate as a measure of physiological arousal) is significantly less strong in children with SCT, compared to their typically developing peers. It is important that parents and professionals working with children with SCT are aware of the potential discrepancies between these two and that it may require a different approach in stimulating the development of emotion regulation skills in these children.
- Studying early development in children with SCT showed that differences in self-regulatory skills compared to typically developing peers can be identified as early as 12 months of age. It also showed that with increasing age, problems in the area of self-regulation may become more pronounced, warranting a developmental approach both in the study of this genetic

condition as well as in clinical care. It is possible that children with SCT show age-appropriate neurocognitive skills at a certain age, but may present serious developmental vulnerabilities at a later age: Continuous monitoring of (neurocognitive) development should thus be considered standard care in children with SCT.

- A neurocognitive approach that examines the underlying building blocks of behavior is helpful in understanding the nature of behavioral problems and individual differences in maladaptive behavior of children with SCT. The impact of the X and Y chromosome on development is likely associated with a suboptimal brain development and broad maturation processes in individuals with SCT, influencing the neurocognitive functioning of the brain that contributes to cognitive, social-emotional, and behavioral problems.

In sum, whilst having an additional X and Y chromosome does not reliably predict an individual's cognitive, emotional, and behavioral outcome, children with SCT evidently are at risk for vulnerabilities in self-regulation that appear more pronounced with increasing age. A developmental neurocognitive perspective is key in increasing our knowledge of gene-brain-behavior pathways in children with SCT as well as advancing clinical care in terms of diagnostics and treatment. Self-regulation amongst other neurocognitive functions may serve as a valuable target for early, tailor-made interventions to minimize the risk for psychopathology later in life and improving quality of life of individuals with SCT.

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Curriculum Vitae

Kimberly Kuiper werd geboren op 9 augustus 1990 te Haarlem. Na het behalen van haar vwo diploma aan de KSH in 2008, begon zij aan de bachelor Pedagogische Wetenschappen aan de Universiteit Leiden waarna zij aansluitend de tweejarige Research Master 'Developmental Psychopathology in Education and Child Studies' met genoegen afrondde bij de afdeling Orthopedagogiek in 2013. Gedurende de Research Master heeft Kimberly een klinische stage doorlopen op het Ambulatorium (spreekuur *neuropedagogiek*), verbonden aan de Universiteit Leiden. Gelijktijdig doorliep Kimberly een onderzoeksstage bij het 'Preventief Interventie Team', waar zij na het behalen van het masterdiploma als onderzoeksmedewerker aan de Universiteit Leiden werkzaam bleef. Naast deze aanstelling was Kimberly betrokken als docent en coördinator binnen de bachelor- en masteropleiding van de afdeling Orthopedagogiek en werkte zij tevens als project onderzoeker bij de Universiteit van Amsterdam. In 2017 startte Kimberly als deelnemer aan de ambitieuze Topklas: een traject waarin zij binnen een periode van zes jaar naast aaneengesloten de opleiding tot gezondheidszorgpsycholoog (GZ) en psychologisch specialist (klinisch neuropsycholoog KNP) tevens werd opgeleid tot klinisch-wetenschappelijk onderzoeker, door het tot stand brengen van een academisch proefschrift. Het centrale thema van het onderzoek was om meer inzicht te krijgen in ontwikkelingsrisico's op het gebied van de zelfregulatie van jonge kinderen met een extra geslachtschromosoom. De resultaten van dit onderzoek staan beschreven in dit proefschrift. Binnen dit onderzoek werkte Kimberly nationaal en internationaal samen met collega onderzoekers en clinici van onder andere de eXtraordinarY Kids Clinic van Children's Hospital Colorado (Denver, Colorado, Verenigde Staten) en was zij samen met haar collega promovendi verantwoordelijk voor de (internationale) data-management en training van (student)onderzoekers. Naast haar aanstelling als promovendus werkte Kimberly tegelijkertijd binnen de specialistische

geestelijke gezondheidszorg (ggz) bij het Leids Universitair Behandel- en Expertisecentrum (LUBEC, voormalig Ambulatorium) van de Universiteit Leiden in verschillende teams. Hier behaalde Kimberly in 2019 haar klinische registraties als GZ-psycholoog en Orthopedagoog-Generalist alvorens zij startte aan de opleiding tot Klinisch Neuropsycholoog. Tijdens haar werkzaamheden droeg Kimberly een significante bijdrage aan het innoveren van het TRIXY Expertisecentrum (bovenregionaal expertisecentrum op het gebied van de X en Y chromosomaal gebonden stoornissen) als ook het vertalen van wetenschappelijke kennis naar de klinische praktijk en patiëntenzorg. Zij verzorgde lezingen, organiseerde samen met collega's een internationaal congres, hielp mee aan het handboek 'Opgroeien met een extra X of Y chromosoom', schreef mee aan brochures en psycho-educatie materiaal voor kinderen, ouders en volwassenen, en was redacteur voor verschillende nieuwsbrieven vanuit het centrum. Hiernaast heeft Kimberly voortdurend een rol gespeeld in het verzorgen van onderwijs in verschillende master- en post-masteropleidingen op het gebied van intelligentie en neuropsychologische diagnostiek en genetische aandoeningen, aan de Universiteit van Leiden als ook de RINO Groep. Op dit moment werkt Kimberly als GZ-psycholoog en Orthopedagoog-Generalist binnen het LUBEC en zal zij haar registratie als klinisch specialist naar verwachting in juni 2023 behalen, waarmee het Topklas traject tot een succesvol einde komt. Kimberly zal haar werkzaamheden, zowel onderzoek als klinisch werk, voortzetten binnen de Universiteit Leiden (TRIXY Expertisecentrum en LUBEC).

List of Publications

Journals

Kuiper, K.C., Swaab, H., Tartaglia, N., & van Rijn, S. (2021). Early developmental impact of sex chromosome trisomies on Attention Deficit-Hyperactivity Disorder symptomology in young children. *American Journal of Medical Genetics Part A*, *185*(12), 3664-3674. https://doi.org/10.1002/ajmg.a.62418

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Van Rijn, S., **Kuiper, K.C.,** Urbanus, E.L., Bouw, J.C., & Swaab, H. (2022). Neurocognitive and behavioral development in young children (1-7 years) with Sex Chromosome Trisomy. *Under review*.

Abstracts

Kuiper, K.C., Bouw, J.C., Swaab, H., & van Rijn, S. (June 24-25, 2017). *Social skills and emotion regulation in young children with sex chromosome trisomy.* AXYS 2017 Family Conference, Denver (CO), USA.

Kuiper, K.C., Urbanus, E.L., Swaab, H. & van Rijn, S. (September 9-10, 2017). *Social attention in young children with sex chromosome trisomies: A case study.* Society for the Study of Behavioral Phenotypes (SSBP) Conference, Leiden, The Netherlands.

Kuiper, K.C., Swaab, H., & van Rijn, S. (September 20-21, 2018). *Social, emotional, and behavioral problems in individuals with Klinefelter Syndrome (47,XXY): A focus on underlying neurocognitive mechanisms.* European Pediatric Psychology Conference (EPPC), Ghent, Belgium.

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Kuiper, K.C., Swaab, H., Tartaglia, N., & van Rijn, S. (September 12-14, 2022). *The developmental impact of SCT on self-regulation skills in early childhood.* Third International Workshop on X and Y Chromosome Trisomies, Leiden, the Netherlands.

Kuiper, K.C., Swaab, H., Tartaglia, N., & van Rijn, S. (November XX, 2022). *Emotion regulation strategies in the context of frustration of young children with SCT.* Nederlandse Vereniging voor Neuropsychologie (NVN), Maastricht, the Netherlands.

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