Neurodevelopmental impact of sex chromosome trisomy in young children: the regulation of emotion, cognition, and behavior

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

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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.