Comparing autism spectrum disorder symptomatology in fragile X syndrome and tuberous sclerosis complex

Melaard, Anoek

MASTER THESIS
CLINICAL NEUROPSYCHOLOGY

Faculty of Behavioural and Social Sciences - Leiden University

Comparing autism spectrum disorder symptomatology in fragile X syndrome and tuberous sclerosis complex

Anoek Melaard - s1869426

October, 2021

External Supervisor: Kyra Lubbers, KJPP Centre of Expertise ENCORE, Erasmus Medical Centre
First Examiner: Karin van der Hiele, Health, Medical and Neuropsychology Unit; Leiden University

Abstract

Introduction: Autism spectrum disorder (ASD) is one of the most prevalent neurodevelopmental disorders and has a major genetic heterogeneity. Comparing monogenetic causes of ASD can contribute to understanding its genetic aetiology. This study compares patients with fragile X syndrome (FXS) to patients with tuberous sclerosis complex (TSC) in terms of ASD severity and symptomatology to create an image of their ASD symptom profiles. This could lead to more focused diagnoses and specialized treatment for these patient groups.

Methods: This study included children and adolescents (ages 1–18, mean age = 9.01 years) with FXS (n = 57, 80.7% males) or TSC (n = 105, 49.5% males). The second editions of the Autism Diagnostic Observation Scale and the Social Responsiveness Scale were used to assess ASD symptomatology. First, the prevalence of official ASD diagnoses among FXS and TSC patients was compared. Next, patients with an official diagnosis were compared in terms of (1) overall autism severity, (2) severity of problems within the restricted and repetitive behaviour (RRB) and social affect domains, and (3) more specific ASD symptoms such as 'reciprocal communication'. Intelligence quotient (IQ; mean IQ = 58.08) was included as a predictor in the logistic regression and as a covariate in both between-group analyses of covariance and all multivariate analyses of covariance.

Results: FXS patients are more likely to receive an official ASD diagnosis (χ^2 = 4.081, p = .043). Higher IQ is related to less autism symptomatology (χ^2 = 8.592, p = .003). Overall, ASD severity of patients with an official ASD diagnosis does not differ between the patient groups. The FXS patients with ASD exhibited more severe RRB (F = 8.21, p = .005). No significant difference was found for social affect or any specific symptoms.

Conclusion: ASD prevalence is higher in children with FXS than in children with TSC, with FXS patients exhibiting more severe RRB. This study illustrates the relevance of comparing symptomatology in monogenetic causes of ASD, indicating that syndrome-symptom relationships can be found. This could lead to earlier intervention and focused treatment for these patients and contributes to research on the genetic aetiology of ASD.

Lekensamenvatting

Inleiding: Autisme spectrum stoornis (ASS) is een veelvoorkomende ontwikkelingsstoornis met diverse (genetische) oorzaken. Deze studie vergelijkt autisme symptomen tussen kinderen met het fragiele X syndroom (FXS) en kinderen met tubereuze sclerose complex (TSC). In de praktijk zien we verschillen in symptomen van autisme bij deze groepen. Deze kunnen veroorzaakt worden door verschillende genetische processen en vereisen wellicht specifiekere behandelmethoden.

Methoden: In deze studie deden kinderen en adolescenten (1 t/m 18 jaar) met FXS of TSC mee. We gebruikten een observatieschema (de ADOS-2) en een vragenlijst (de SRS-2) om symptomen van autisme te meten. De ADOS-2 werd uitgevoerd door een onderzoeker en de SRS-2 werd ingevuld door de ouders/verzorgers van het kind. Bij het vergelijken van de groepen hebben we rekening gehouden met mogelijke verschillen in ontwikkelingsniveau.

Eerst onderzochten we in welke groep de meeste officiële ASS-diagnoses voorkwamen. Daarna vergeleken we de kinderen met een officiële ASS-diagnose, om te kijken hoe ernstig hun autisme symptomen waren. Daarbij keken we naar twee overkoepelende kenmerken van autisme: 'restrictief en repetitief gedrag' en 'sociaal affect' (dit heeft betrekking op communicatie en sociale interactie). Tot slot onderzochten we enkele specifieke symptomen zoals 'denkbeeldig spel'.

Resultaten: We vonden dat kinderen met FXS een hogere kans hebben op een officiële ASS-diagnose dan kinderen met TSC, maar dat de ernst van autisme niet verschilt tussen de groepen met een officiële diagnose. De kinderen met FXS en ASS hadden meer problemen met 'restrictief en repetitief gedrag' dan kinderen met TSC en ASS, maar de groepen verschilden niet in de mate van problemen met 'sociaal affect' en ook niet op de specifieke symptomen zoals 'denkbeeldig spel'. Daarbij behaalden de kinderen die ook ASS hadden een lagere score op een IQ test.

Conclusie: We concluderen dat ASS meer voorkomt bij kinderen met FXS dan bij kinderen met TSC en dat kinderen met FXS en ASS meer problemen lijken te ervaren met 'restrictief en repetitief gedrag'. Deze studie laat zien dat de symptomen van autisme kunnen verschillen tussen stoornissen. Dit kan ons meer leren over de relatie tussen genetische afwijkingen en bepaalde autistische symptomen. Bovendien helpt dit ons gerichter te kunnen behandelen.

Table of contents

1. Introduction

Autism spectrum disorder (ASD) is one of the most prevalent neurodevelopmental disorders and is significantly influenced by genetics (Sandin et al., 2017). The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013) defines autism as a spectrum disorder because ASD has been shown to be a complex behavioural syndrome with varying severity levels and multiple aetiologies. The identified core elements are impairments in social interaction and restrictive and repetitive behaviour (RRB; Budimirovic & Kaufmann, 2011). The increase in research on ASD genetics indicates its massive heterogeneity with over 1,000 genes being related to ASD (Thapar & Rutter, 2020). This genetic heterogeneity complicates the search for the aetiological cause of ASD. This study aims to contribute to this search by comparing two genetically homogenous groups with a high prevalence of co-occurring syndromic autism: fragile X syndrome (FXS) and tuberous sclerosis complex (TSC). Studying ASD in syndromes with a known genetic cause could identify pathways underlying idiopathic ASD phenomenology. In addition, it could reveal syndrome-specific symptomatology, which can be used for early and targeted intervention. Finally, since intelligence quotient (IQ) influences behaviour and both groups have a high prevalence of intellectual disability, this study also takes the role of intelligence into account (Côté et al., 2020).

1.1 Fragile X syndrome

FXS is the most common monogenic cause of inherited intellectual disability and ASD (Niu et al., 2017). It is caused by an expansion of over 200 CGG nucleotide sequence repeats in the fragile X mental retardation gene on the X chromosome. Males are more often and more severely affected by the disorder than females because females have a protective unaffected X chromosome (Loesch, Huggins & Hagerman, 2004). The CGG expansion causes a loss of fragile X mental retardation 1 protein, resulting in intellectual disability. Additionally, FXS is associated with many behavioural abnormalities, of which autism is one of the most severe (Kaufmann et al., 2004; McDuffie et al., 2015). Among males with FXS, 90% display autistic features, and 60% meet the diagnostic criteria for ASD (McDuffie, Thurman, Hagerman & Abbeduto, 2015). ASD diagnosis and autistic behaviours are relatively stable during the lifespan of males with FXS (Hernandez et al., 2009).

The ASD symptom profile of FXS patients can differ from those of patients with nonsyndromic ASD. Behavioural phenotypes of the two groups have similarities, such as social interaction deficits (Kaufmann et al., 2004), persistent gaze avoidance (Roberts et al., 2007), delay in understanding language and severe social indifference (Budimirovic & Kaufmann,

2011). However, the symptoms often seem to be less severe in FXS patients (Budimirovic & Kaufmann, 2011; Niu et al., 2017). FXS patients seem to experience more problems with RRB than with social interaction and communication (McDuffie et al., 2015; Wheelers et al., 2015). The autistic features in FXS patients are likely driven by impairments in complex social behaviour, and these impairments in interaction with peers set them apart from FXS patients without ASD (Hernandez et al., 2009). ASD symptomatology in FXS patients could also be (partly) explained by their lower intelligence and frequent comorbid anxiety that contributes to impairments in social behaviour (Budimirovic & Kaufmann, 2011; Côté et al., 2020).

1.2 Tuberous sclerosis complex

TSC is a rare autosomal-dominant neurocutaneous disorder caused by mutations of either the TSC1 or TSC2 gene. It is characterized by benign tubers that almost always affect the central nervous system, leading to epilepsy and neuropsychiatric disorders (Curatolo, Bombardieri & Jozwiak, 2008). Commonly observed neurodevelopmental disorders include autism, which is prevalent in 40 to 50% of TSC patients (Curatolo, Napolioni & Moavero, 2010). Over the last decade, researchers have begun to investigate the prevalence and identifiable biomarkers in more depth to predict autism in TSC (Debopam, 2020). The interrelationship between TSC and ASD is complex. Researchers have found that patients with TSC and ASD often have an earlier onset of epilepsy and more frequent seizures than TSC patients without ASD. However, little knowledge exists regarding the neurobiological link between epilepsy and ASD and therefore the interaction between the two is unclear (Specchio et al., 2020).

A study from Jeste and colleagues (2016) compared toddlers with TSC and ASD to toddlers with nonsyndromic ASD. The profile of social communication impairment, including gestures, eye contact, shared enjoyment, pointing, and responsive smiling was strongly comparable between the two groups, indicating that TSC patients with ASD have substantial problems with social interaction. Problems with RRB among TSC patients with ASD has been studied less. It could be that many TSC patients meet some of the ASD characteristics but do not qualify for a full diagnosis when RRB is not present (Capal et al., 2018).

1.3 Research objectives and implications

The aim of this study is to compare the ASD symptomatology of FXS patients and patients with TSC. Despite the high comorbidity of autism in both disorders and ongoing research, the exact profile of ASD symptoms, the role of intellectual disability and the underlying mechanisms are still unclear (Niu et al., 2017; Specchio et al., 2020). Both disorders

have been compared to nonsyndromic ASD (e.g., Abbeduto et al., 2019; Jeste et al., 2016). However, this is one of the first studies to compare them to each other. A study by Côté et al. (2020) included both groups but focused on the influence of behavioural issues on adaptive functioning rather than on ASD symptomatology. This study aims to obtain a clearer image of ASD symptom profiles within these patient groups. This could lead to more focused diagnoses and to the development of specialized treatment for children with FXS or TCS who also struggle with ASD symptoms. Greater treatment efficacy would benefit these patients with comorbid ASD and is currently lacking for both groups (Abbeduto et al., 2019; Debopam, 2020). Furthermore, comparing symptomatology in monogenetic disorders could help associate certain ASD symptoms with genetic defects. Therefore, this study could aid in further understanding the aetiology of ASD.

1.4 Hypotheses

The first hypothesis is that ASD prevalence is higher in FXS patients than in TSC patients since FXS is the most common monogenic cause of inherited ASD (Curatolo, Napolioni & Moavero, 2010; McDuffie et al., 2015; Niu et al., 2017). In addition, ASD severity is compared between FXS and TSC patients with an official ASD diagnosis. Only patients with an official diagnosis are compared to account for the expected difference in ASD prevalence. Since it is expected that FXS patients are more likely to receive an official diagnosis, it is expected that their overall symptoms are more prominent. Therefore, hypothesis 2 states that the FXS group is more severely affected in the case of an official diagnosis. To further specify the differences between the two groups, symptom severity is then compared in terms of the two core elements of autism: RRB and social affect and communication (SA).

For the RRB domain, FXS patients seem to experience problems similar to those of nonsyndromic ASD patients (McDuffie et al., 2015; Wheelers et al., 2015). Studies comparing TSC patients to patients with nonsyndromic ASD have yielded unclear results for RRB severity in TSC patients, suggesting less severe RRB symptoms in TSC patients than in nonsyndromic ASD patients (Capal et al., 2018). Therefore, hypothesis 3 predicts that FXS patients with ASD are more severely affected in the RRB domain than TSC patients with ASD are.

For the SA domain, TSC patients have demonstrated a profile of social communication impairment that is very similar to that of nonsyndromic ASD patients (Jeste et al., 2016). FXS groups have demonstrated fewer SA impairments compared to nonsyndromic ASD groups (McDuffie et al., 2015; Niu et al., 2017). Therefore, hypothesis 4 states that FXS patients with ASD will be less severely affected in the SA domain than TSC patients with ASD.

Finally, although the total scores and the scores in the RRB and SA domains are informative, they do not include all information of the measurement instruments. Analysing the subscales of second editions of the Autism Diagnostic Observation Scale (ADOS-2) and the second edition of the Social Responsiveness Scale (SRS-2) can provide additional information about the patients' autism profiles. These subscales represent ASD symptoms such as 'reciprocal communication' and 'imaginary play'; more extensive explanation of the subscales is provided in Section 2.3. Hypothesis 5 states that ADOS-2 subscales C (play), D (stereotypic behaviour and limited interests) and E (other behaviour, e.g., anxiety) are more severely affected in FXS patients with ASD, while subscales A (language and communication) and B (reciprocal social interaction) are more severe in TSC patients with ASD. Hypothesis 6 states that SRS-2 subscales A (consciousness), B (cognition), C (communication) and D (motivation) are more severely affected in TSC patients with ASD, while subscale E (preoccupation) is more affected in FXS patients with ASD. These hypotheses are based on the partial overlap of some subscales with the RRB and SA domains and in line with hypotheses 3 and 4.

2. Methods

2.1 Design

This is a cross-sectional study in which children with FXS are compared to children with TSC in ASD symptomatology and its severity. The data used in this study was collected through the VOLG (*Vroege Onderkenning van Lichamelijke en Leer-/Gedragsproblemen bij kinderen met erfelijke neuro-cognitieve aandoeningen*) study from the centre of expertise ENCORE (*Erfelijke Neuro-cognitieve Ontwikkelingsstoornissen Rotterdam Erasmus MC*) in Rotterdam, the Netherlands. The VOLG study is a prospective longitudinal observational study that investigates social, behavioural and cognitive development of children with rare genetic disorders. The VOLG study is still in process, but enough data has been collected to provide a substantial base for this study.

The Medical Ethical Testing Commission (METC) of the Erasmus Medical Centre has approved the VOLG research project and stated that no informed consent from the participants and/or their parents was needed (METC 2017-529, approved on April 4th, 2015). Nevertheless, informed consent was requested from all parents of children who are under the age of 16 and/or not mentally competent to make their own decisions. Young adolescents from ages 12 to 15 provided informed consent in addition to their parents' consent. Adolescents over the age of 16 provided informed consent. The research is in conformity with the Declaration of Helsinki and its later amendments (World Medical Association, 2013).

2.2 Participants

The data used in the current study was collected between September 2013 and October 2020. The participants are patients seen at the ENCORE expertise centre within the Erasmus MC-Sophia Children's Hospital in Rotterdam, the Netherlands. The data used in this study comes from an existing database of data collected during regular clinical care. Therefore, the participants did not have to be recruited for this study specifically. The database includes information from 310 participants, including 82 FXS and 228 TSC patients. All FXS and TSC patients could be included regardless of their level of intelligence and responsivity. When a participant had multiple measurements, the most recent or most complete one was selected. In addition, participants with missing IQ data were excluded. After exclusion, the FXS group consisted of 46 males and 11 females aged 1 to 18 (mean (SD) age: 8.28 (4.44) years) and the TSC group of 52 males and 53 females aged 1 to 18 (mean (SD) age: 9.40 (4.58) years). We expected there to be relatively more males than females in the FXS group since males are more often and more severely affected by FXS (Loesch, Huggins & Hagerman, 2004).

2.2.1 Selecting participants with autism spectrum disorder. To account for the difference in ASD prevalence between FXS and TSC patients and to specify the symptoms of patients who are classified as on the autism spectrum, only patients with an official ASD diagnosis were included for further analyses of symptomatology. The participant selection process for the various analyses is illustrated in Figure 1. Patients were selected based on official ASD diagnoses since this was deemed more reliable than selection based on one-time measurements obtained from the ADOS-2 and SRS-2. Diagnoses were given by a psychiatrist and/or multidisciplinary team based on (long-term) information gathered during clinical care using the guidelines of the DSM-5 (American Psychiatric Association, 2013). In short, the DSM-5 ASD criteria state that a patient must suffer from (1) persistent deficiencies in social communication and interaction in various situations and (2) restricted or repetitive behaviour and/or interests. In addition, the symptoms must have been present from a young age and interfere with daily functioning. Finally, the symptoms must not be better explained by intellectual disabilities or a developmental delay.

Figure 1

Participant selection process
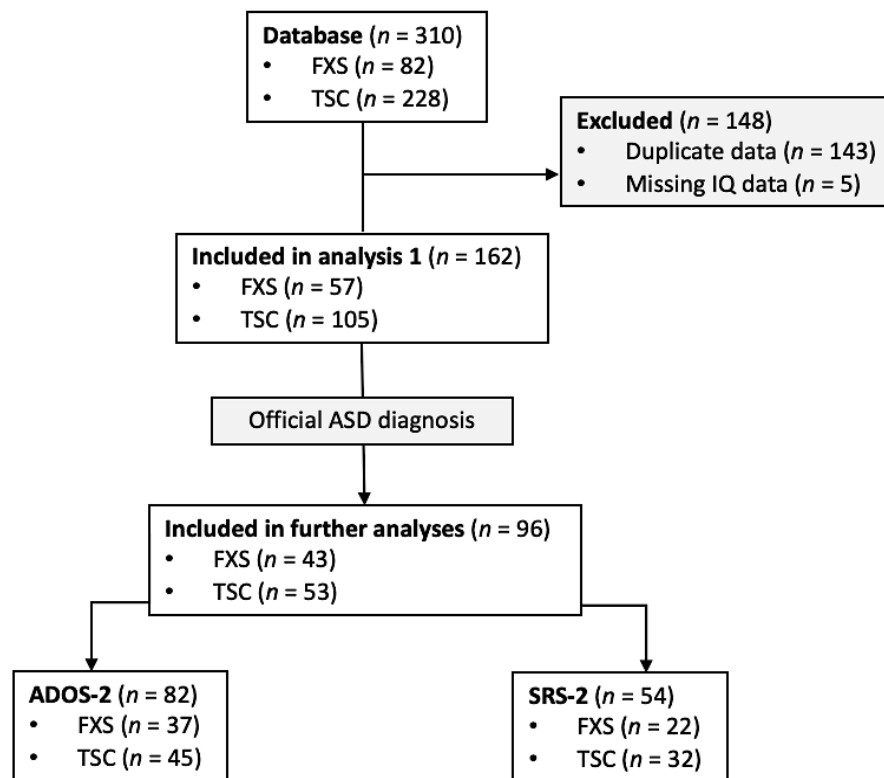

```
                    ┌─────────────────────────────┐
                    │ Database (n = 310)          │
                    │  •   FXS (n = 82)           │
                    │  •   TSC (n = 228)          │
                    └─────────────────────────────┘
                              │        ┌──────────────────────────────────┐
                              │───────▶│ Excluded (n = 148)               │
                              │        │  •   Duplicate data (n = 143)    │
                              │        │  •   Missing IQ data (n = 5)     │
                              │        └──────────────────────────────────┘
                    ┌─────────────────────────────┐
                    │ Included in analysis 1 (n = 162) │
                    │  •   FXS (n = 57)           │
                    │  •   TSC (n = 105)          │
                    └─────────────────────────────┘
                              │
                    ┌─────────────────────────────┐
                    │ Official ASD diagnosis      │
                    └─────────────────────────────┘
                              │
                    ┌─────────────────────────────────────┐
                    │ Included in further analyses (n = 96)│
                    │  •   FXS (n = 43)                   │
                    │  •   TSC (n = 53)                   │
                    └─────────────────────────────────────┘
                       │                          │
         ┌────────────────────────┐    ┌────────────────────────┐
         │ ADOS-2 (n = 82)        │    │ SRS-2 (n = 54)         │
         │  •   FXS (n = 37)      │    │  •   FXS (n = 22)      │
         │  •   TSC (n = 45)      │    │  •   TSC (n = 32)      │
         └────────────────────────┘    └────────────────────────┘
```

Note: FXS = fragile X syndrome; TSC = tuberous sclerosis complex; ADOS-2 = Autism Diagnostic Observation Scale, second edition; SRS-2 = Social Responsiveness Scale, second edition

2.3 Measures

Two reliable measurements of autism severity were included in this study, namely the ADOS-2 (De Bildt, Graeves-Lord & De Jonge, 2019) and the SRS-2 (Constantino & Gruber, 2012). Both instruments have five subscales that analyse different ASD symptoms and partly correspond to the RRB domain and the SA domain. They both provide a total severity score and two severity scores for the RRB and SA domains separately. The SRS-2 provides insight into a child's functioning in their daily environment, providing information complementary to the ADOS-2, which is administered in a test setting. The instruments have a high convergent validity, and together they can improve the rate of correct ASD indications (Constantino & Gruber, 2012; Medda, Cholemkery & Freitag, 2018).

2.3.1 The Autism Diagnostic Observation Scale (second edition). The ADOS-2 is a semi structured observational measurement in which a trained examiner presents the participant with a series of activities and materials. These activities and materials can elicit ASD symptoms, which allows for the observation of symptoms in both the RRB and SA domains

(Abbeduto, 2020; Hus, Gotham & Lord, 2012). Both domains are calculated by an algorithm that uses the scores achieved on five subscales: (A) language and communication; (B) reciprocal social interaction; (C) play; (D) stereotypic behaviour and limited interests; and (E) other behaviour including observed hyperactivity, anxiety, or disrupted behaviour. Items scored on each subscale can receive a score from 0 to 3, where 0 indicates normal behaviour and 3 indicates severe behavioural problems. A score of 7 or 8 is given when an item cannot be scored (e.g., the item 'echolalia' cannot be scored for nonverbal participants). The ADOS-2 scoring manual is then used on these 'raw' scores to create scores that can be statistically analysed, meaning that scores of 1 remain 1, scores of 3 and higher are changed to 2, and scores of 7 or 8 (i.e., items that could not be scored) are changed to 0. The ADOS-2 then provides calibrated severity scores (CSS) for the overall severity of ASD symptoms, for the RRB domain and for the SA domain. These CSS can range from 1 to 10 (Hus, Gotham & Lord, 2021). Certain social interaction and communication scores of subscales A and B are included in the SA CSS, while several scores on subscale D are included in the RRB CSS. Subscales C and E are not included in any of the CSS.

The ADOS-2 is available in five modules, each of which is applicable to different groups based on chronological age and level of expressive language (Hus, Gotham & Lord, 2012). Module-T is applied to toddlers with no phrase speech, module 1 to children with no phrase speech, module 2 to children with (nonfluent) phrase speech, module 3 to verbally fluent children and module 4 to adolescents and adults who can communicate verbally. The sensitivity and specificity of the ADOS-2 differ per module and lie between 72 and 97% and 19 and 94%, respectively (Gotham, Risi, Pickles & Lord, 2007). The ADOS-2 modules contain different items and cannot be compared directly. To allow for comparisons between modules, the raw scores are transformed to individual divided scores. The raw scores are obtained by adding the scores of the scoring manual (e.g., the 0s, 1s and 2s) per subscale for each participant. The individual divided scores are then calculated by dividing a participant's raw score on a subscale by the maximum obtainable score on that subscale. These individual divided scores lay between 0 and 1 and allow for comparison between ADOS-2 modules. Item A1 was deleted from analysis since it determines whether an ADOS-2 module is suitable for a participant and does not measure any ASD symptoms.

2.3.2 The Social Responsiveness Scale (second edition). The SRS-2 is a questionnaire completed by the child's parent(s) or caregiver(s). It consists of 65 items that measure autism severity in everyday life (Constantino & Gruber, 2012). It provides indications about a child's social impairments, social consciousness, social information processing and social anxiety

and/or avoidance (Bruni, Constantino & Gruber, 2014). Each item can be scored from 1 to 4, with higher scores indicating a higher severity of ASD symptoms. These relate to five subscales: (A) consciousness (i.e., the ability to comprehend social cues), (B) cognition (i.e., the ability to interpret social cues), (C) communication (i.e., reciprocal communication), (D) motivation (i.e., motivation to participate in social environments) and (E) preoccupation (i.e., RRB). Subscales A, B, C and D correspond to the SA domain, while subscale E corresponds to the RRB domain (Constantino & Gruber 2012). SRS-2 T-scores above 75 indicate severe ASD symptoms and a strong association with an official ASD diagnosis. T-scores between 61 and 75 indicate moderate and scores between 60 and 65 mild deficiencies in reciprocal social behaviour. T-scores below 59 are not associated with clinical ASD and are considered typical (Constantino & Gruber, 2012). The SRS-2 is available in four forms corresponding to different age groups. This study includes the Preschool Form (applicable to ages 2.5 to 4.5) and the School-Age Form (for ages 4 to 18). The sensitivity and specificity of the SRS-2 are 93 and 91%, respectively (Kidd et al., 2019).

  2.3.3 Intelligence. Finally, since this study also takes the role of IQ into account, age- and developmentally-appropriate IQ tests are included, specifically the third and fifth edition of the Wechsler Intelligence Scale for Children (Wechsler, 1991, 2018), the third edition of the Wechsler Preschool and Primary Scale of Intelligence (Wechsler, 2002), the Wechsler Nonverbal Scale of Ability (Wechsler, 2006), the Sneijders-Oomen Non-Verbal Intelligence Test (Telleggen et al., 2005), and the third edition of the Bayley Scales of Infant and Toddler Development (Weiss, Oakland & Aylward, 2010). One of these intelligence tests was selected for each participant based on their estimated developmental level. For all Wechsler intelligence scales and the Sneijders-Oomen Non-Verbal Intelligence Test, a total IQ score can be calculated. In the case of the Bayley Scales of Infant and Toddler Development, a developmental quotient is calculated by dividing developmental age by chronological age. This developmental quotient can be interpreted as a total IQ score (Weiss, Oakland & Aylward, 2010).

2.4 Procedure

  The data used in this study was collected during regular clinical care. As far as possible, participants underwent the same testing procedures every few years for follow-up purposes, thus providing data for both cross-sectional and longitudinal research. Properly trained researchers administered a standardized battery of (neuro)psychological tests, including the ADOS-2 and age-appropriate intelligence tests. The parent(s) or caregiver(s) were asked to

complete the questionnaires online. As previously stated, the METC found that informed consent was not needed for data analysis. Nevertheless, all parents and (mentally competent) adolescents ages 12 and up were asked to provide informed consent, and only those who did were included in our analyses.

2.5 Statistical analyses

To test hypothesis 1, all patients with available IQ data were included. A logistic regression was used to assess the impact of the group (FXS or TSC) and IQ on the likelihood of an official ASD diagnosis. To test hypothesis 2, two between-group analyses of covariance (ANCOVAs) were performed to compare the overall severity of ASD symptoms between the two groups. Here, only the children with an official ASD diagnosis were included. The dependent variable was either the total CSS of the ADOS-2 or the total T-score of the SRS-2, and IQ was included as the covariate. Preliminary assumption testing was conducted to check for normality, linearity, homogeneity of variance, homogeneity of regression slopes and reliable measurement of the covariate. No serious violations were noted.

To test hypotheses 3 and 4 concerning the RRB domain and SA domain respectively, two multivariate analyses of covariance (MANCOVA) were performed to analyse the domains. Again, only children with an official ASD diagnosis were included. Since not all participants had obtained reliable data from both instruments, one MANCOVA was performed for the ADOS-2 and another for the SRS-2 data. The dependent variables were the severity scores in the RRB and SA domains. IQ score was included as a covariate in both analyses. Preliminary assumption testing was conducted to check for normality, linearity, univariate and multivariate outliers, homogeneity of variance-covariance matrices and multicollinearity, with no serious violations noted.

To test hypotheses 5 and 6 concerning the individual subscales of the ADOS-2 and the SRS-2, two MANCOVAs were performed. In the case of the SRS-2, the raw scores were transferred to T-scores, added per subscale and then compared between groups. In the case of the ADOS-2, the raw scores were transferred to individual divided scores to allow for comparisons among all ADOS-2 modules. IQ was again included as a covariate in both analyses. Preliminary assumption testing was conducted to check for normality, linearity, univariate and multivariate outliers, homogeneity of variance-covariance matrices and multicollinearity, with no serious violations noted.

3. Results

3.1 Descriptive statistics

The sample consisted of 162 children and adolescents with a mean age of 9.01 years (standard deviation (SD) = 4.55). The sample included 57 FXS patients (mean age = 8.28, SD = 4.44, 80.7% male, 75.4% with an official ASD diagnosis) and 105 TSC patients (mean age = 9.4, SD = 4.58, 50.5% male, 50.5% with an official ASD diagnosis). In total, 146 participants completed a module of the ADOS-2 (module-T: n = 3; module 1: n = 51; module 2: n = 32; module 3: n = 50; module 4: n = 14), and 96 participants had complete SRS-2 data. The descriptive statistics are summarized in Table 1.

Table 1

Participant demographics

	FXS	FXS + ASD	TSC	TSC + ASD	Total
Participants (*n*)	57	43	105	53	162
Gender (male %)	80.7%	81.4%	49.5%	54.7%	60.5%
Mean age (SD)	8.28 (4.44)	9.14 (4.31)	9.40 (4.58)	9.77 (1.45)	9.01 (4.55)
Mean IQ (SD)	46.37 (18.73)	45.93(17.08)	64 (29.61)	45.93(17.08)	58.08 (27.63)

Note: FXS = fragile X syndrome; ASD = autism spectrum disorder; TSC = tuberous sclerosis complex; *n* = number of participants; SD = standard deviation

3.2 Official autism spectrum disorder diagnosis

A logistic regression was performed to assess the impact of group (FXS or TSC) and IQ on the likelihood of an official ASD diagnosis. The full model containing both predictors was statistically significant (χ^2 = 19.203, p < .001). It explained between 11.2% (Cox and Snell R square) and 15.1% (Nagelkerke R square) of the variance in ASD diagnosis and correctly classified 72.2% of the cases. Both independent variables made a unique significant contribution to the model. The strongest predictor was IQ, where a higher IQ relates to a lower chance of an official ASD diagnosis. Group was also a significant predictor; being part of the FXS group was associated with a higher chance of having an official ASD diagnosis than being part of the TSC group was. The findings are summarized in Table 2.

Table 2

Influence of intelligence quotient and fragile X syndrome or tuberous sclerosis complex diagnosis in an official autism spectrum disorder diagnosis

	B	**SE**	**Wald χ^2**	**p**	**Exp (B)**	**95% CI**
Group (FXS vs. TSC)	0.779	.386	4.081	.043*	2.179	4.640
IQ	-0.200	.007	8.592	.003**	.981	.994

Note: ASD = autism spectrum disorder; FXS = fragile X syndrome; TSC = tuberous sclerosis complex; B = unstandardized regression weight; SE = standard error; *p* = probability; Exp (B) = odds ratio; CI = confidence interval; * = *p* < .05; ** = *p* < .01

3.3 Overall autism severity

Two ANCOVAs were conducted to compare the overall ASD severity of FXS patients and TSC patients with an official ASD diagnosis. The first ANCOVA included the total CSS of the ADOS-2 as the dependent variable, and the second included the total SRS-2 T-score as the dependent variable. IQ was included as a covariate in both analyses. Neither the ADOS-2 nor the SRS-2 scores differed significantly between the two groups (ADOS-2: F (1, 82) = .94, *p* = .36, partial η^2 = .01; SRS-2: F (1, 51) = 3.24, *p* = .08, partial η^2 = .06). Both analyses revealed a relationship between IQ and total severity score as indicated by a partial η^2 value of .18 and .14 for the ADOS-2 and the SRS-2, respectively. A higher IQ score correlated with a lower total severity score. Table 3 displays the mean scores and SDs of both groups' total severity scores of the ADOS-2 and SRS-2.

Table 3

Total severity scores on the Autism Diagnostic Observation Scale (second edition) and the Social Responsiveness Scale (second edition) for patients with an official autism spectrum disorder diagnosis

	FXS + ASD			**TSC + ASD**			
	n	*M*	SD	*n*	*M*	SD	*p*
ADOS-2[CSS]	38	6.25[a]	.38[a]	47	5.75[a]	.34[a]	.36
	38	6.47[u]	2.19[u]	47	5.57[u]	2.82[u]	
SRS-2[T]	22	89.81[a]	3.12[a]	32	82.48[a]	2.58[a]	.08
	22	91.00[u]	13.17[u]	32	81.66[u]	16.77[u]	

Note: FXS = fragile X syndrome; ASD = autism spectrum disorder; TSC = tuberous sclerosis complex; ADOS-2 = Autism Diagnostic Observation Scale, second edition; [CSS] = calibrated severity score; SRS-2 = Social Responsiveness Scale, second edition; [T] = T-score; [a] = adjusted mean (controlling for the covariate IQ); [u] = unadjusted mean; *n* = number of participants; *M* = mean score; SD = standard deviation; *p* = probability

3.4 Restricted and repetitive behaviour and social affect and communication

To test hypotheses 3 and 4 that FXS patients with ASD are more severely affected in the RRB domain and less severely affected in the SA domain than TSC patients with ASD, two MANCOVAs were performed to analyse the RRB and SA domains of both the ADOS-2 and the SRS-2. The first MANCOVA included the CSS of the RRB and SA domain of the ADOS-2 as dependent variables and the second the T-scores of both domains on the SRS-2. IQ was included as a covariate in both analyses. For the ADOS-2, there was a statistically significant difference between the groups in terms of the combined dependent variables, F (2, 78) = 4.17, p = .02; Wilks' λ = .90; partial η^2 = .097. When the results for the dependent variables were considered separately, the only difference to reach statistical significance using a Bonferroni adjusted alpha level of .025 was in the RRB domain, F (1, 79) = 8.21, p = .005; partial η^2 = .09. An inspection of the mean scores indicated that FXS patients with ASD scored moderately higher in the RRB domain (*M* = 6.91, SD = .38) than TSC patients with ASD (*M* = 5.45, SD = .34). Similar results were found for the SRS-2, where a significant difference was found only for the RRB domain, F (1, 51) = 10.13, p = .002; partial η^2 = .166. An inspection of the mean scores indicated that FXS patients with ASD scored higher in the RRB domain (*M* = 87.76, SD = 2.71) than TSC patients with ASD (*M* = 76.51, SD = 2.24). Both analyses also revealed a relationship between IQ and RRB and SA severity scores as indicated by a partial η^2 value of .26 and .30 for the ADOS-2 and the SRS-2, respectively. A higher IQ score correlated to a lower total severity score. The findings are summarized in Table 4 and illustrated in Figures 2 and 3.

Table 4

Mean scores in the restrictive and repetitive behaviour and social affect and communication domains for patients with an official autism spectrum disorder diagnosis

	FXS + ASD			TSC + ASD			
	n	*M*	SD	*n*	*M*	SD	*p*
ADOS-2[CSS]							
RRB	37	6.91	.38	45	5.45	.34	.005*
SA	37	6.24	.38	45	6.21	.34	.952
SRS-2[T]							
RRB	22	87.76	2.71	32	76.51	2.24	.002*
SA	22	79.40	3.09	32	76.73	2.55	.511

Note: FXS = fragile X syndrome; ASD = autism spectrum disorder; TSC = tuberous sclerosis complex; ADOS-2 = Autism Diagnostic Observation Scale, second edition; [CSS] = calibrated severity score; SRS-2 = Social Responsiveness Scale, second edition; [T] = T-score; RRB = restricted and repetitive behaviour; SA = social affect and communication; *n* = number of participants; *M* = mean score; SD = standard deviation; *p* = probability; * = $p < .025$

Figure 2

Boxplots of Autism Diagnostic Observation Scale, second edition total-, RRB- and SA- calibrated severity scores
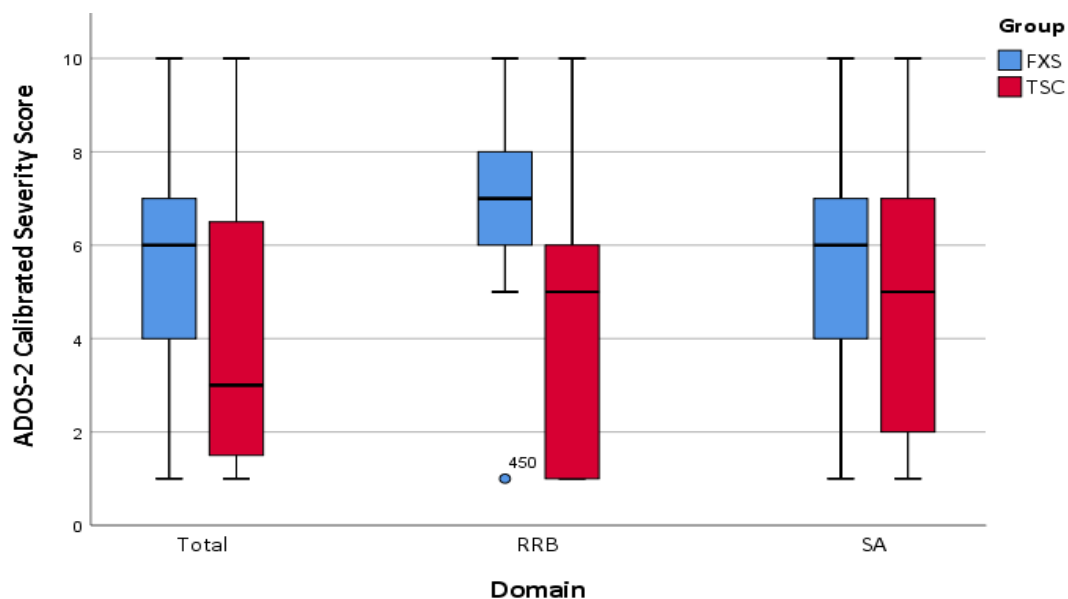
Note: ADOS-2 = Autism Diagnostic Observation Scale, second edition; RRB = restricted and repetitive behaviour; SA = social affect; FXS = fragile X syndrome; TSC = tuberous sclerosis complex

Figure 3

Boxplots of Social Responsiveness Scale (second edition) T-scores on the total Social Responsiveness Scale (Second Edition) and in the restricted and repetitive behaviour and social affect and communication domains
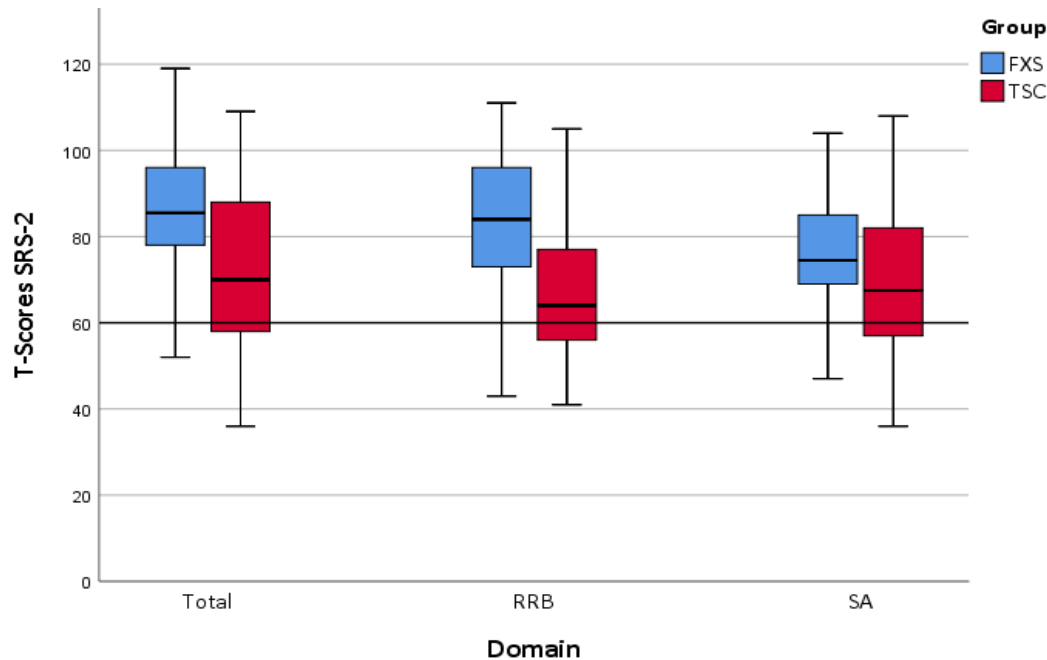
Note: The horizontal line at 60 indicates the minimal T-score for ASD-related symptoms. SRS-2 = Social Responsiveness Scale, second edition; RRB = restricted and repetitive behaviour; SA = social affect; FXS = fragile X syndrome; TSC = tuberous sclerosis complex

3.5 Specific symptomatology

Two more MANCOVAs were performed for the individual subscales of the ADOS-2 and SRS-2. IQ was included as a covariate in both analyses. The first MANCOVA included the weighted averages from all subscales of the ADOS-2 as dependent variables. There was a statistically significant difference between the groups in the combined dependent variables, F (1, 85) = 2.40, p = .04; Wilks' λ = .88; partial η^2 = .13. However, when the results for the dependent variables were considered separately using a Bonferroni adjusted alpha level of .01, no significant difference was discovered in any of the subscales. The second MANCOVA included the T-scores from all subscales of the SRS-2 as dependent variables. There was no statistically significant difference between the groups in the combined dependent variables, F (1, 51) = 1.99, p = .098; Wilks' λ = .83; partial η^2 = .17. Therefore, the subscales were not investigated further. The findings are summarized in Table 5 and illustrated in Figures 4 and 5.

Table 5

Mean scores on Autism Diagnostic Observation Scale (second edition) and Social Responsiveness Scale (second edition) subscales for patients with an official autism spectrum disorder diagnosis

	FXS + ASD		TSC + ASD		
	M	SD	*M*	SD	*p*
ADOS-2[IDS]	(*n* = 40)		(*n* = 47)		
A: Language and communication	.40	.18	.32	.17	.086
B: Reciprocate social interaction	.51	.22	.45	.26	.685
C: Play	.43	.39	.54	.66	.247
D: Stereotypic behaviour	.37	.20	.25	.25	.070
E: Other behaviour	.27	.22	.23	.30	.373
SRS-2[T]	(*n* = 22)		(*n* = 32)		
A: Consciousness	73.59	11.99	68.72	16.24	.424
B: Cognition	83.82	11.43	77.56	15.59	.235
C: Communication	85.36	13.42	78.19	16.87	.209
D: Motivation	77.82	14.98	72.41	16.12	.265
E: Preoccupation	89.14	12.37	75.56	15.09	.002*

Note: FXS = fragile X syndrome; ASD = autism spectrum disorder; TSC = tuberous sclerosis complex; ADOS-2 = Autism Diagnostic Observation Scale, second edition; [IDS] = individual divided score; SRS-2 = Social Responsiveness Scale, second edition; [T] = T-score; *n* = number of participants; *M* = mean score; SD = standard deviation; *p* = probability; * = *p* < .01

Figure 4

Boxplots of Autism Diagnostic Observation Scale (second edition) individual divided scores per
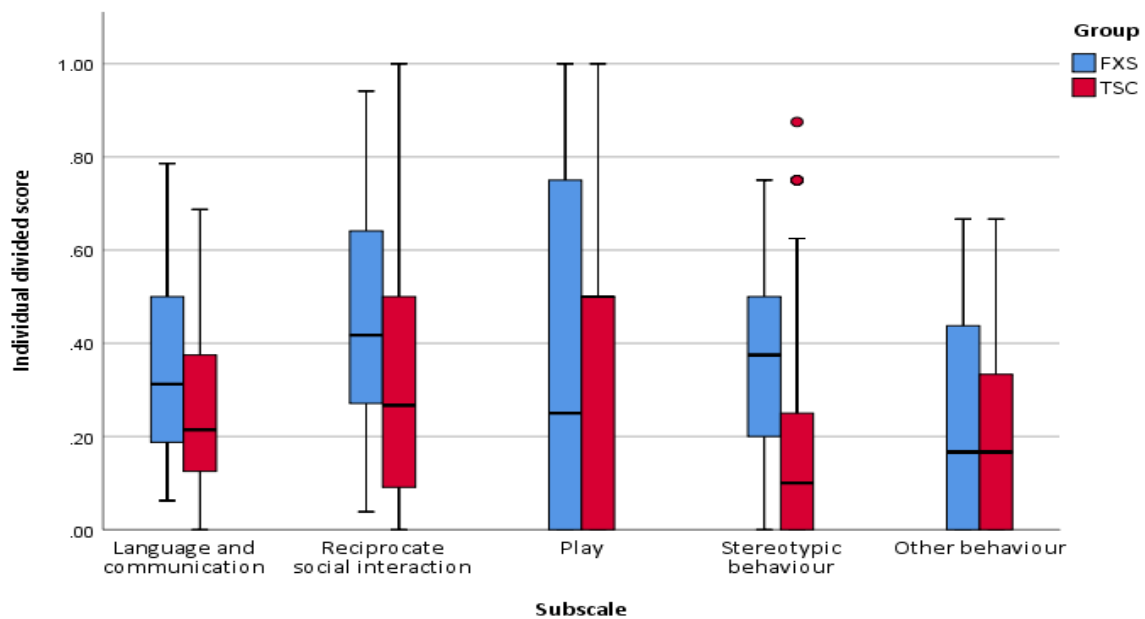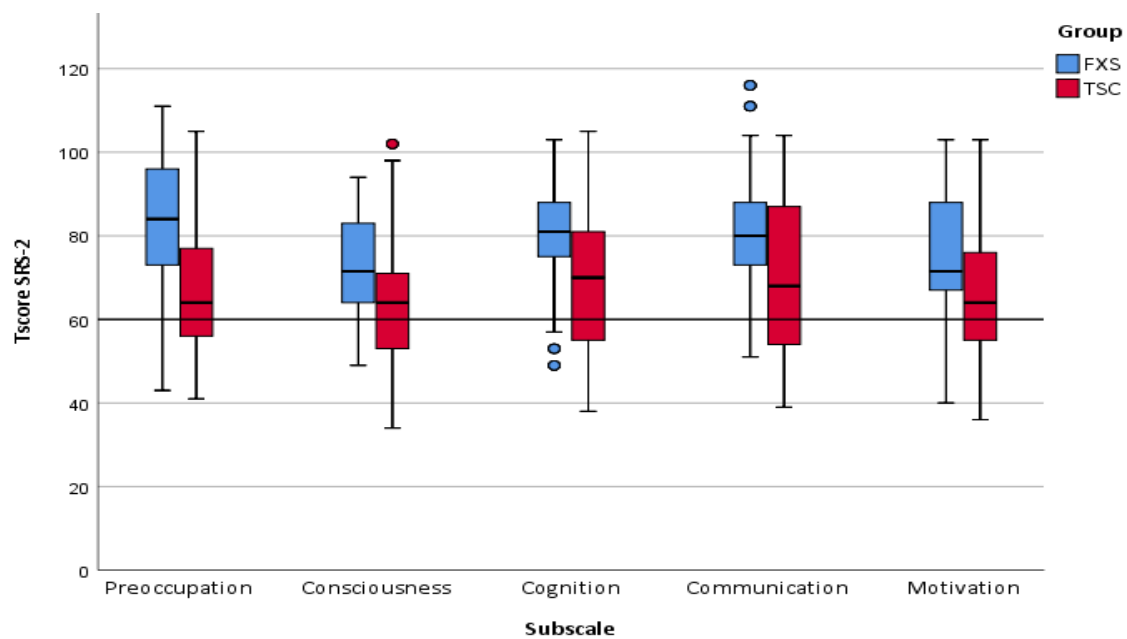

subscale for both groups

Note: ADOS-2 = Autism Diagnostic Observation Scale, second edition; FXS = fragile X syndrome; TSC = tuberous sclerosis complex

Figure 5

Boxplots of Social Responsiveness Scale (second edition) T-scores per subscale for both groups

Note: The horizontal line at 60 indicates the minimal T-score for ASD-related symptoms. SRS-2 = Social Responsiveness Scale, second edition; FXS = fragile X syndrome; TSC = tuberous sclerosis complex

4. Discussion

        This study was intended to produce a clearer picture of ASD symptom profiles among FXS and TSC patients. This could lead to earlier and more specific interventions for FXS and TCS patients with comorbid ASD. In addition, this study is intended to aid in the further understanding of the genetic aetiology of ASD by associating certain ASD symptoms with these monogenetic causes. This study found that FXS patients were more likely to have an official diagnosis of ASD than TSC patients were. However, no significant difference was found between the two groups in overall ASD severity. When investigating the RRB and SA domains separately, the FXS patients with ASD were found to be more severely affected in the RRB domain than TSC patients with ASD were. This was found using both the ADOS-2 and the SRS-2 and suggests that there might be a difference in ASD symptomatology between the groups. Finally, no significant differences were found for the SA domain or any of the individual subscales of either of the instruments.

4.1 Differences in autism spectrum disorder prevalence and the role of intelligence

        In line with hypothesis 1, it was found that FXS patients were more likely to have an official ASD diagnosis than patients with TSC were (Curatolo et al., 2010; McDuffie et al., 2015). In addition, IQ partly accounted for ASD severity as indicated by the lower severity scores after correction for IQ (see Table 3). This is in line with earlier findings that IQ relates to the severity of ASD symptoms of the RRB and SA domains (Bishop, Richler & Lord, 2006; Hirosawa et al., 2020). Bishop, Richler and Lord (2006) studied the relationship between nonverbal IQ and RRB problems in 830 children with ASD and found a negative relationship for most of the RRB behaviours. A follow-up study confirmed that RRB can be subdivided into 'repetitive sensory motor behaviours' and 'insistence on sameness behaviours' and that IQ only has a negative relation with repetitive sensory motor behaviours (Bishop et al., 2012). This indicates that a higher IQ is related to a lower severity of most RRB symptoms but not all.

        Hirosawa and colleagues (2020) compared the influence of IQ on social cognition between children with ASD and typically developing children using the SRS. They found that higher intelligence was associated with better social cognition in children with ASD but not in typically developing children. They propose that in ASD patients, a higher intelligence compensates for persistent deficits in social cognition and/or that it is used for successful learning, while in typically developing children, social cognition is an automatic process. This finding highlights the need to consider intelligence when studying ASD and when using the SRS as method of ASD measurement.

4.2 Differences in overall autism spectrum disorder severity

Since FXS patients were expected to be more likely to have an official ASD diagnosis, it was hypothesized that overall, they would be more severely affected by ASD symptoms than TSC patients would be. However, no significant difference in overall ASD severity between the groups was found. Only patients with an official ASD diagnosis were included. Since the DSM-5 acknowledges that autism is a spectrum disorder with three severity levels, it was expected that differences in overall severity could be found (American Psychiatric Association, 2013). However, it is possible that both FXS and TSC patients are at the lower end of the spectrum since both FXS and TSC patients have been demonstrated to be less severely affected than patients with nonsyndromic ASD (Budimirovic & Kaufmann, 2011; Niu et al., 2017; Capal et al., 2018). The difference in ASD severity between FXS and TSC patients on the spectrum could be too small to classify as a different severity level according to the DSM-5.

4.3 Differences in autism spectrum disorder symptomatology

4.3.1 Restricted and repetitive behaviour domain. As hypothesized, the RRB domain was found to be more severely affected in FXS patients with ASD than in TSC patients with ASD. Measurements with both the ADOS-2 and the SRS-2 showed this. Since these instruments provide complementary information about ASD functioning and have a high convergent validity; a valid difference in ASD symptomatology between the groups is indicated (Constantino & Gruber, 2012; Medda, Cholemkery & Freitag, 2018). Further research is needed to confirm these findings, but the possibility of specifying ASD symptom profiles within these patient groups seems feasible. This would mean that more focussed treatment (i.e., treatment for FXS patients that targets problems with RBB) could be established.

4.3.2 Social affect and communication domain. Contrary to our expectations, no difference was found between the two groups in SA severity. Hypothesis 4, concerning the SA domain, was based on the similarities of the social communication profile found between TSC patients with ASD and nonsyndromic ASD patients (Jeste et al., 2016) and the less affected SA domain in FXS patients with ASD compared to nonsyndromic ASD patients (McDuffie et al., 2015; Niu et al., 2017). It is not ideal to base hypotheses on studies that do not compare the disorders directly since the results are not fully comparable. Future studies are encouraged to compare monogenetic disorders directly and to include typically developing and nonsyndromic ASD control groups. The study of Côté et al. (2020) sets a fitting example. Adding a nonsyndromic ASD control group creates a baseline to compare ASD symptoms to and makes it easier to compare results to existing literature.

4.3.3 Individual subscales. Finally, the individual subscales were not further investigated since no significant difference was found in the combined dependent variables. When considering the probability levels in Table 5, it becomes apparent that FXS patients with ASD score significantly higher in 'stereotypic behaviour' and 'preoccupation'. However, these subscales do not provide additional information because they are identical to the RRB domain. Nevertheless, we argue that subscale analyses can reveal strengths and weaknesses that would be overlooked if only the total severity scores were investigated. The findings imply that the ASD symptom profiles of FXS and TSC patients with ASD are quite similar, indicating that similar treatments could be used for these patient groups. Future researchers are encouraged to study more monogenetic disorders with a high comorbidity of ASD and to investigate the individual subscales to reveal syndrome-specific symptom profiles.

4.4 Strengths and limitations

4.4.1 Strengths. This study is the first to directly compare ASD symptomology between children and adolescents with FXS and TSC. By showing that problems with RRB are more related to FXS, a link between the monogenetic cause of FXS and RRB symptoms is indicated. This demonstrates the value of comparing monogenetic disorders when investigating ASD aetiology. In addition, a strength of this study was including both the ADOS-2 and SRS-2. Combining these two instruments constitutes a promising, reliable measurement of ASD severity for future research. Including both instruments provides information about behaviour in an observational setting as well as in daily life, creating a realistic view of autistic functioning. By studying the subscales of both tests, a specifically focused view on symptomatology can be created. Future researchers are encouraged to use these instruments in combination to study ASD symptoms in various patient groups.

Another key strength of this study is including IQ as a covariate, thereby taking into account one of the main confounding factors influencing ASD severity (Budimirovic & Kaufmann, 2011; Bishop, Richler & Lord, 2006; Hirosawa et al., 2020). However, IQ is not the only factor influencing ASD severity. Attention deficit hyperactivity disorder, anxiety and gender are all factors that should be taken into consideration, especially for these patient groups (Budimirovic & Kaufmann, 2011; Debopam, 2020). Including all of these factors as confounding factors can be a challenge since the exact interaction or overlap between them remains unclear and requires further investigation (Côté et al., 2020; Specchio et al., 2020).

4.4.2 Limitations. A limitation of this study is including only patients with an official ASD diagnosis while analysing symptomatology. This was decided to account for the difference

in ASD prevalence between the two groups and to specify ASD symptoms of individuals who are on the spectrum. However, Wheelers et al. (2015) found that significantly fewer FXS patients were diagnosed with ASD based on the DMS-5 criteria than would have been diagnosed based on behavioural symptom criteria endorsed by caregivers. They found that most FXS patients met the DSM-5 criteria for ASD in the RRB domain but not in the SA domain. Their study suggests that adjusting the DSM-5 criteria for the SA domain by one symptom could lead to an increase in official ASD diagnoses for FXS patients. Similarly, the prevalence of official ASD diagnoses in the TSC group could increase if criteria for the RRB domain were less strict (Capal et al., 2018). Therefore, one could argue that this study neglected patients with comorbid ASD symptoms by only including those with an official diagnosis. Indicating whether patients who do not qualify for an official diagnosis still experience certain ASD symptoms could be beneficial for clinical use and create treatment possibilities (Capal et al.,2018; Wheelers et al., 2015). Therefore, future studies should consider including all patients regardless of an official diagnosis and use the ADOS-2 and SRS-2 to indicate ASD symptomatology. This could also increase sample sizes, although it will be challenging to assemble much larger samples when researching rare monogenetic disorders. The samples in this study were relatively large, but all participants were patients seen at the ENCORE expertise centre within the Erasmus MC-Sophia Children's Hospital, which could potentially lead to a selection bias towards a clinical sample. The inclusion of typically developing and nonsyndromic ASD control groups should be considered to increase sample sizes and create a baseline measurement.

4.5 Conclusion

Comparing ASD symptomatology in monogenetic disorders seems to be a promising method for further understanding the genetic aetiology of ASD. This study concludes that ASD prevalence is higher in children with FXS than in children with TSC. Differences between the groups in symptomatology are found in the RRB domain, which seems to be more severely affected in FXS patients with ASD. This finding emphasizes the relevance of comparing symptomatology in monogenetic causes of ASD because it indicates that syndrome-specific symptomatology could become apparent, which is useful for early and targeted intervention. Future studies are encouraged to include typically developing and nonsyndromic ASD control groups and to make comparisons with other monogenetic disorders. This would allow for further investigations into possible syndrome-symptom relationships and, as a consequence, for more targeted treatments for patient groups with comorbid ASD.

References

Abbeduto, L., Thurman, A. J., McDuffie, A., Klusek, J., Feigles, R. T., Brown, W. T., Harvey, D. J., Adayev, T., LaFauci, G., Dobkins, C., & Roberts, J. E. (2019). ASD comorbidity in fragile X syndrome: Symptom profile and predictors of symptom severity in adolescent and young adult males. *Journal of Autism and Developmental Disorders, 49*(3), 960-977. doi: 10.1007/s10803-018-3796-2

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Author, Arlington, VA

Bishop, S. L., Hus, V., Duncan, A., Huerta, M., Gotham, K., Pickles, A., Kreiger, A., Buja, A., Lund, S., & Lord, C. (2013). Subcategories of restricted and repetitive behaviours in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 43*, 1287-1297. doi: 10.1007/s10803-012-1671-0

Bishop, S. L., Richler, J., & Lord, C. (2006). Association between restricted and repetitive behaviors and nonverbal IQ in children with autism spectrum disorders. *Child Neuropsychology, 12*(4), 247-267. doi: 10.1080/09297040600630288

Bruni, T. P., Constantino, J. N., & Gruber, C. P. (2014). Social Responsiveness Scale-Second Edition (SRS-2). *Journal of Psychoeducational Assessment, 32*(4), 365-369. doi: 10.1177/073428913517525

Budimirovic, D. B., & Kauffmann, W. E. (2011). What can we learn about autism from studying fragile X syndrome? *Developmental Neuroscience, 33*(5), 379-394. doi: 10.1159/000330213

Capal, J. Horn, P., Kent, B., Weber, A. W., Bing, N., Pearson, D., Sahin, M., & Krueger, D. (2018). The broader autism phenotype in tuberous sclerosis complex. *Neurology, 90*(15).

De Bildt, A., Graeves-Lord, K., & De Jonge, M. (2019). *ADOS-2 Autisme diagnostisch observatieschema*. Hogrefe.

Constantino, J. N., & Gruber, C. P. (2012). *Social Responsiveness Scale, Second Edition*. Los Angeles, CA: Western Psychological Services.

Côté, V., Knoth, I. S., Lalancette, E., Lavergne, J. A., Côté, L., Major, P., & Lippé, S. (2020). Behavioural characteristics related to adaptive functioning in young persons with tuberous sclerosis complex, Down syndrome and fragile X syndrome. *Journal of Developmental and Physical Disabilities.* doi: 10.1007/s10882-020-09748-8

Curatolo, P., Bombardieri, R., & Jozwaik, S. (2008). Tuberous sclerosis. Lancet, 327(9639), 657-668. doi: 10.1016/S0140-6736(08)61279-9

Curatolo, P., Napolioni, V., & Moavero, R. (2010). Autism spectrum disorders in tuberous

sclerosis: Pathogenetic pathways and implications for treatment. *Journal of Child Neurology, 25*(7), 873-880. doi: 10.1177/0883073810361789

Debopam, S. (2020). An update review of tuberous sclerosis complex-associated autism spectrum disorder. *Pediatric Neurology, 109*, 4-11. doi: 10.1016/j.pediatrneurol.2020.03.008

Gotham, K., Risi, S., Pickles, A., & Lord, C. (2007). The Autism Diagnostic Observation Schedule: Revised algorithms for improved diagnostic validity. *Journal of Autism and Developmental disorders, 37*(4), 613-627. doi: 10.1007/s10803-006-0280-1.

Hernandez, R. N., Feinberg, R. L., Vaurio, R., Passanante, N. M., Thompson, R. E., & Kaufmann, W. E. (2009). Autism spectrum disorder in fragile X syndrome: a longitudinal evaluation. *American Journal of Medial Genetics Part A, 149A*(6), 1125-1137. doi: 10.1002/ajmg.a.32848

Hirosawa, T., Kontani, K., Fukai, M., Kameya, M., Soma, D., Hino, S., Kitamura, T., Hasegawa, C., An, K. M., Takahashi, T., Yoshimura, Y., & Kikuchi, M. (2020). Different associations between intelligence and social cognition in children with and without autism spectrum disorders. *Plos One, 15*(8). doi: 10.1371/journal.pone.0235380

Hus, V., Gotham, K., & Lord, C. (2012). Standardizing ADOS domain scores: Separating severity of social affect and restricted and repetitive behaviours. *Journal of Autism and Developmental Disorders, 44*(10), 2400-2412. doi: 10.1007/s10803-012-1719-1

Jeste, S. S., Varcin, K. J., Hellemann, G. S., Gulsrud, A. C., Bhatt, R., Kasari, C., Wu, J. Y., Sahin, M., & Nelson, C. A. (2016). Symptom profiles of autism spectrum disorder in tuberous sclerosis complex. *Neurology, 87*(8), 766-772. doi: 10.1212/WNL.0000000000003002

Kaufmann, W. E., Cortell, R., Kau, C. S. M., Bukelis, I., Tierney, E., Gray, R. M., Cox, C., Capone, G. T., & Stanard, P. (2004). Autism spectrum disorder in fragile X syndrome: Communication, social interaction, and specific behaviors. *American Journal of Medical Genetics Part A, 129A*(3), 225-234. doi: 10.1002/ajmg.a.30229

Kidd, S. A., Berry-Kravis, E., Choo, T. H., Chen, C., Esler, A., Hoffmann, A., Andrews, H. F., & Kaufmann, W. E. (2020). Improving the diagnosis of autism spectrum disorder in fragile X syndrome by adapting the Social Communication Questionnaire and the Social Responsiveness Scale-2. *Journal of Autism and Developmental Disorders, 50*(9), 3276-3295). doi: 10.1007/s10803-019-04148-0

Loesch, D. Z., Huggins, R. M., & Hagerman, R. J. (2004). Phenotypic variation and FMRP levels in fragile X. *Mental Retardation and Developmental Disabilities Research Reviews, 10*(1), 31-41. doi: 10.1002/mrdd.20006

McDuffie, A., Abbeduto, L., Lewis, P., Kover, S., Kim J. S., Weber, A., & Brown, W. T. (2010). Autism Spectrum Disorder in children and adolescents with fragile X syndrome: Within-syndrome differences and age-related changes. *American Journal on Intellectual and Developmental Disabilities, 115*(4), 307-326. doi: 10.1352/1944-7558-115.4.307

McDuffie, A., Thurman, A. J., Hagerman, R. J., Abbeduto, L. (2015). Symptoms of autism in males with fragile X syndrome: A comparison to nonsyndromic ASD using current ADI-R scores. *Journal of Autism and Developmental Disorders*.

Medda, J. E., Cholemkery, H., & Freitag, C. M. (2018). Sensitivity and Specificity of the ADOS-2 algorithm in a large German sample. *Journal of Autism and Developmental Disorders, 49* (2), 750-761. doi: 10.1007/s10803-018-3750-3

Niu, M. M., Han, Y., Dy, A. B. C., Du, J. B., Jin, H. F., Qin, J., Zhang, J., Li, Q. R., &   Hagerman, R. J. (2017). Autism symptoms in fragile X syndrome. *Journal of Child Neurology, 32*(10), 903-909. doi: 10.1177/0883073817712875

Roberts, J. E., Weisenfeld, L. H., Hatton, D. D., Heath, M., & Kaufmann, W. E., (2007). Social approach and autistic behavior in children with fragile x syndrome*. Journal of Autism and Developmental Disorders, 37*(9), 1748-1760.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Hultman, C., Larsson, H., & Reichenberg, A. (2017). The heritability of autism spectrum disorder. Journal of the American Medical Association, 318(12), 1182-1184. doi: 10.1001/jama.2017.12141

Specchio, N., Pietrafusa, N., Trivisano, M., Moavero, R., De Palma, L., Ferretti, A., Vigevano, F., & Curatolo, P. (2020). Autism and epilepsy in patients with tuberous sclerosis complex. *Frontiers in Neurology, 11*(639). doi: 10.3389/fneur.2020.00639

Tellegen, P., Winkel, M, Wijnberg-Williams, B., Laros, J. A. (2005). *Snijders–Oomen Niet-Verbale Intelligentietest 2,5–7: Handleiding.* Boom Testuitgevers.

Thapar, A., & Rutter, M. (2020). Genetic advances in autism. *Journal of Autism and Developmental Disorders*. doi: 10.1007/s10803-020-04685-z

Wechsler, D. (1991). *The Wechsler Intelligence Scale for Children* (3rd ed.). The Psychological Corporation.

Wechsler, D. (2018). *Wechsler Intelligence Scale for Children* (5th ed.). Amsterdam: Pearson Benelux B.V.

Wechsler, D. (2002). *Wechsler Preschool and Primary Scale of Intelligence* (3rd ed.). The Psychological Corporation.

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

Assessment.

Weiss, L. G., Oakland, T., & Aylward, G. P. (2010). *Bayley-III Clinical Use and Interpretation*. Academic Press. doi: 10.1016/C2009-0-01670-5

Wheeler, A. C., Mussey, J., Villagomez, A., Bishop, E., Raspa, M., Edwards, A., Bodfish, J., Bann, C., & Bailey, D. B. (2015). DSM-5 changes and the prevalence of parent-reported autism spectrum symptoms in Fragile X syndrome. *Journal of Autism and Developmental Disorders, 45*(3), 816-29. doi: 10.1007/s10803-014-2246-z

World Medical Association (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA, November 2013, 310*(20): 2191-4. doi: 10.1001/jama.2013.281053