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Autism Spectrum Disorder traits in children and young people with Fragile X Syndrome: A Systematic Review

&

A comparison of Behavioural Phenotypes of Autism Spectrum Disorder traits in boys and girls with Fragile X Syndrome.

Thesis submitted in partial fulfilment of the requirement for the degree of:

Doctorate of Clinical Psychology (DClinPsy)

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Cardiff University

Dale Thomas

Supervised by: Dr Dougal Hare

20/05/2019

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This thesis is being submitted in partial fulfilment of the requirements for the degree of ... (*insert PhD, MD, MPhil, etc., as appropriate*)

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This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is it being submitted concurrently for any other degree or award (outside of any formal collaboration agreement between the University and a partner organisation)

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(Excluding summary, acknowledgements, declarations, contents pages, appendices, tables, diagrams and figures, references, bibliography, footnotes and endnotes)

Summary of thesis

Three papers are presented as a thesis for partial fulfilment for the Doctorate in Clinical Psychology (DClinPsy). The first two are presented as papers for submission to the Journal of Autism and Developmental Disorders (JADD), which presents papers related to Autism Spectrum Disorders (ASD) and Developmental Disabilities. The third paper is a reflective paper on the process of undertaking the former two. This is not prepared for publication.

Paper 1 is a systematic review of the methodological quality of extant research of 'co-morbid' Autism Spectrum Disorder in children and young people with Fragile X Syndrome. It reviews 13 methodologically sound peer reviewed papers and presents a qualitative synthesis of findings. The synthesis highlights the direction of impairment of particular ASD behavioural phenotypes and a trajectory model for the development of ASD traits in FXS.

Paper 2 presents an empirical study of gender differences in FXS and comorbid FXS and ASD (FXS+ASD) presentations, also considering the impact of intellectual disability. The study utilised an online platform and accessed participants via the Fragile X Society database. Participants were parents of children with a diagnosis of FXS. They completed a series of questionnaire measures, which indicated the sample did not differ significantly according to gender; severity of ASD traits was not associated with cognitive ability; and the presence of ASD traits increased with age. The latter finding supported the trajectory model suggested in Paper 1.

Paper 3 provides a considered critique and reflective evaluation of the process of undertaking the previous papers. It provides a personal iterative reflection into the impact of the author's prior experiences and the impact of these on the direction of the research thesis. As well as additional

reflection on challenges and limitations of the research process; cumulating in highlighting the transformative nature of the research process and the plan for dissemination of findings.

Acknowledgments

This doctoral thesis is dedicated in loving memory of David Thomas. Pa, you taught me more than any book ever will. You are the heart of the family and you are with us every step we take.

To my awesome children, Lewis, Ava and Xavier. You are the life that runs through me and the energy that drives me. On days when I needed a lift, a water fight, a beach fire, a surf or a good old fashioned *cwtch* was always on hand. You make me smile every day. I am so proud to be your Pa. Love you all to the moon and back. P.S. I'll be out of the shed soon.

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Autism Spectrum Disorder traits in children and young people with Fragile X Syndrome: A Systematic Review

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(Prepared for Journal of Autism and Developmental Disorders)

(See Appendix A)

Abstract

Background: *To evaluate whether the high percentage of individuals with Fragile X Syndrome (FXS) who demonstrate comorbid Autism Spectrum Disorder (ASD) symptoms display behavioural phenotypes (BPs) of a categorically distinct disorder to FXS.*

Methods: A literature search of MEDLINE, PsychINFO and Embase was conducted of English language, peer reviewed journals. Studies of the BP of FXS in participants aged 0-25years, with a genetically confirmed presentation of FXS and a clinically diagnosed ASD control or reference group were included.

Results: Thirteen studies were reviewed (n=1,319) that considered research questions exploring the BP of FXS and ASD. Study design included longitudinal, as well as cross sectional cohort studies, assessing gender differences, intellectual ability, presentation of early infants and older children.

Discussion*: A* distinction between FXS and FXS with comorbid ASD exists. This is proposed to be on a continuum of impairment, the direction of impairment depends on the individual BP, and has been termed FXS+. A trajectory model of development is supported.

Key words: Fragile X Syndrome; FXS; Autism Spectrum Disorder; ASD; children; comorbid

Introduction

People with Fragile X Syndrome (FXS) often present with autistic traits (Hagerman et al., 1986). However, there is no consensus as to whether Autism Spectrum Disorder (ASD) in FXS is categorically distinct (Cornish, Turk & Levitas, 2007; Hall., Lightbody, Hirt, Rezvani, & Reiss, 2010; Abbeduto, McDuffie & Thurman, 2014). A comorbid perspective is that symptoms of autism in FXS share the same underlying neurological and psychological dysfunctions as idiopathic ASD (iASD). Alternatively, a continuum view holds that ASD in FXS is the complex and severe end of the FXS range of impairment. To understand these discrepancies distinction between the genetically diagnosed FXS and behaviourally diagnosed ASD is paramount (Halle et al., 2010).

Patterns of behaviour that present in syndromes caused by chromosomal or genetic differences are termed behavioural phenotypes (BP). They are characterised by patterns of social, linguistic, cognitive and motor observations, reliably associated with biological or genetic disorders (O'Brien, 2006). Charting complex phenotypical and developmental trajectories can help identify markers of shared aetiology and advance insight into underlying cognitive, neurological and genetic mechanisms; and clarify direct clinical and theoretical implications (Lee, Martin, Berry-Kravis, & Losh, 2016).

Confirmation of BPs is beneficial for recognition, diagnosis, early intervention and evaluation (Cross & Hare, 2013). In FXS delineating BP is theorised to determine how behaviour, genes and cognitive function interact in FXS (Dykens, 2000) and improve understanding of the mechanisms behind genotype expression. Thus, it provides valuable research data for clinical practice in areas such as self-harm, social anxiety, sensory differences, social skills, emotional disturbances and repetitive behaviours (Waite et al., 2014). Recent research into FXS and ASD has progressed and through exploration of the behavioural phenotype, potential differences are emerging (Daffin, Thomas, Hardiman & Hare; In Submission).

Fragile X syndrome

Pre or postnatal genetic testing provides diagnosis of pre-mutation or full mutation FXS. FXS is the most commonly known cause of inherited intellectual disability (Turner, Webb, Wake, & Robinson, 1996; Crawford, Acuña, & Sherman, 2001), affecting approximately 1 in 4,000 males and 1 in 6,000 females (Boyle & Kaufmann, 2010). FXS results from a cytosine-guanine-guanine (CGG) expansion that triggers hyper-methylation and silencing of the FMR1 on the X chromosome at Xq27.3. This creates a direct correlation in the number of CGG repeats in the sequence and informs severity of the phenotype. The expansion leads to a decrease or absence of the fragile X mental retardation protein (FMRP) produced by the FMR1 gene. Expansions in the 60–200 repeat status, termed pre-mutation or individuals termed carriers, do not significantly affect the transcription of FMRP. But expansions above 200 CGG repeats lead to the full mutation in their offspring (O'Brien, 2006).

Individuals with the FMR1 pre-mutation do not generally have the same BP as full mutation FXS (Boyle & Kaufmann, 2010). As X-linked disorder, males with FXS tend to be more impaired than females due to females having one X chromosome that carries a healthy FMR1 allele. Nearly all males with FXS will have an intellectual disability (ID) but only a third of females (Hagerman et al., 2009; Gallagher & Hallahan, 2012; Crawford, Moss, Anderson, Oliver & McCleery, 2015). Males with FXS-O (FXS without co-morbid ASD) generally present with mild ID (Full Scale IQ; FSIQ 55-70), whereas males with co-morbid FXS+ASD usually have moderate ID, FSIQ 40-54 (Boyle & Kaufmann, 2010). Hardiman and Bratt (2016) have begun to explore the prevalence of stress-related circuits that may inform the BP of FXS presenting as exaggerated behavioural responses to stressors. Consequently, the hypothalamic-pituitary adrenal (HPA) axis, a stress effector system that triggers the release of cortisol, is hypothesised to be an important agent in social and psychological behaviours. Given the

close association between FXS and ASD, it is suggested that children with ASD experience increased cortisol response to stress (Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006; Spratt et al., 2012) and that reduced FMRP may result in excessive activation of HPA axis (Hessl et al., 2002). Exploration of the role of HPA axis in the development of a BP in FXS has gathered evidence for the efficacy that cortisol levels differ in FXS compared to typically developing (TD) controls (Hardiman & Bratt. 2016). Cordeiro, Ballinger, Hagerman and Hessl (2011) report 86% of individuals with FXS have an anxiety disorder, with comorbid anxiety leading to increased impairment.

Autism spectrum disorder

Autism spectrum disorder is characterised by difficulty in communication, reciprocal social interaction and restrictive, repetitive stereotyped behaviours (American Psychiatric Association, 2013). National Institute of Health and Clinical Excellence (NICE, 2011; updated 2017) guidance recommends that diagnosis of ASD is made according to the Diagnostic Statistics Manual (DSM-V; American Psychiatric Association, 2013) or International Classification of Diseases version 10 (ICD10; World Health Organization, 1992) criteria. NICE indicate a gold standard assessment should include standardised measures such as the Autism Diagnosis Observation Schedule (ADOS; Lord et al., 1989; Lord et al, 2000); the Autism Diagnostic Interview – Revised (ADI-R; Lord et al. 1994); or the Diagnostic Instrument for Social and Communication Disorders (DISCO; Wing, Leekam, Libby, Gould, & Larcombe, 2002).

Many children with FXS typically present with marked behavioural features that can include attention deficits; hyperactivity; hyper arousal; anxiety; repetitive behaviours/interests, vocalisations and gestures; social difficulties and gaze avoidance similar to those observed in children diagnosed with

iASD. Consequently, the assessment of comorbid ASD in children with FXS is increasingly undertaken (Hall, et al., 2010).

Relationship between FXS and ASD

Evidence that certain individuals with genetic and metabolic syndromes might have an atypical profile of ASD phenomenology is emerging, supporting a distinction between syndromic variants of ASD and iASD (Moss & Howlin, 2009; Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010). More than 90% of males with FXS display behaviours that are similar to those observed in individuals with iASD (Bailey, Hatton, Mesibov, Ament & Skinner, 2000). Feinstein and Reiss (1998) proposed that there may be a genetic explanation for this, with a genetically distinct subgroup of individuals with FXS. Despite this, only a small portion meet full DSM-IV criteria for autistic disorder (Budimirovic and Kaufmann, 2011) and only 25% of girls and boys receive a diagnosis in clinical practice (Klusek, Martin & Losh, 2014). As the presence and degree of ASD symptoms differs, considerable variability is observed in the behavioural phenotype of FXS (Abbeduto, McDuffie & Thurman, 2014). ASD is heterogenous in nature, confounding assessment and formulation of specific phenotypical behaviour and subsequent diagnosis. Furthermore, cognitive abilities are more limited in individuals with FXS than in individuals with iASD, with more than 90% of males with FXS having an IQ in the range of ID (Hessl et al., 2009). UK rates of ASD are around 1 in 100 (Baird et al., 2006) and 1-68 in the USA (Baio, 2014). Prevalence rates of FXS individuals proposed to have comorbid ASD fluctuate in research findings from: 21-51% (Moss & Howlin, 2009); 22-33% (Zafeiriou, Ververi, Dafoulis, Kalyva, & Vargiami, 2013) and 30% (Richards, Jones, Groves, Moss, & Oliver, 2015).

Research into co-morbid ASD and FXS has historically recruited individuals with a prior diagnosis of ASD, FXS, or co-morbid diagnosis. The ADOS and ADI-R are diagnostic tools but may not provide the sensitivity to understand individual difference in FXS as they are not measuring severity of symptomology. They were not designed, normed or calibrated for FXS and lack specificity in syndrome variances. For instance, in a sample of 63 boys with FXS (aged 2-19) only 24% of the sample met the diagnostic criteria for ASD on ADOS and ADI-R measures and DSM-IV criterion, whereas an additional 44% met the criteria on one or two of the three measures (Harris et al., 2008), thus highlighting the difficulties in applying dichotomous and phenomenologically defined diagnoses to behaviours and symptoms that vary throughout a developmental trajectory (Hall et al., 2010).

FXS with co-morbid conditions appears to increase the quantity and severity of phenotypic behaviours and is associated with lower parental estimates of their child's Quality of Life (QoL; Bailey, Raspa, Olmsted & Holiday, 2008). Hagerman et al. (2017) report an extensive set of studies that present highly related co-occurring issues that if addressed could improve QoL. These include: psychosocial characteristics such as anxiety, ASD symptoms, self-injury and aggression; health problems such as seizures and sleep deprivation; reduced cognitive and executive functioning; and social isolation due to poor functional and adaptive behaviour.

The aim of the current review is to systematically explore the methodological quality and findings of extant research examining BP of 'co-morbid' ASD in children and young people with FXS. In examining the BP, support for either comorbid disorder or continuum hypotheses may emerge. This may provide fundamental information regarding the diagnosis and intervention requirements within this group.

Method

Search strategy

MEDLINE (1946) PsychINFO (1806-) Embase (1947-) databases were searched for relevant articles in October 2018. Searches were limited to those published in English, peer-reviewed journals and empirical study. The following search terms were used with Boolean operators and truncation as indicated, to search in title or abstract: Fragile X Syndrome, fragile x syndrome,. Fxs, "AND" Autism Spectrum Disorders, autism spectrum disorder*, asd, autism, Aspergers syndrome. Followed by a search in all fields for one of: phenotype "OR" phenotypes, behaviour*, children, adolescents, comorbid. intellectual disabilit*"OR" learning disability*.

Selection criteria

The inclusion criteria were: studies of BP of FXS; study participants with FXS provided evidence of genetic testing and participants with ASD had received a clinical diagnosis (confirmed by the study); papers included participants with FXS, or FXS with comorbid ASD; the maximum age of participants was 25 (to match the current education provision range); papers published after 2000 were included; empirical study. The exclusion criteria were: studies focussing on premutation only; single participant case studies; animal studies; studies researching medication intervention only.

Search results

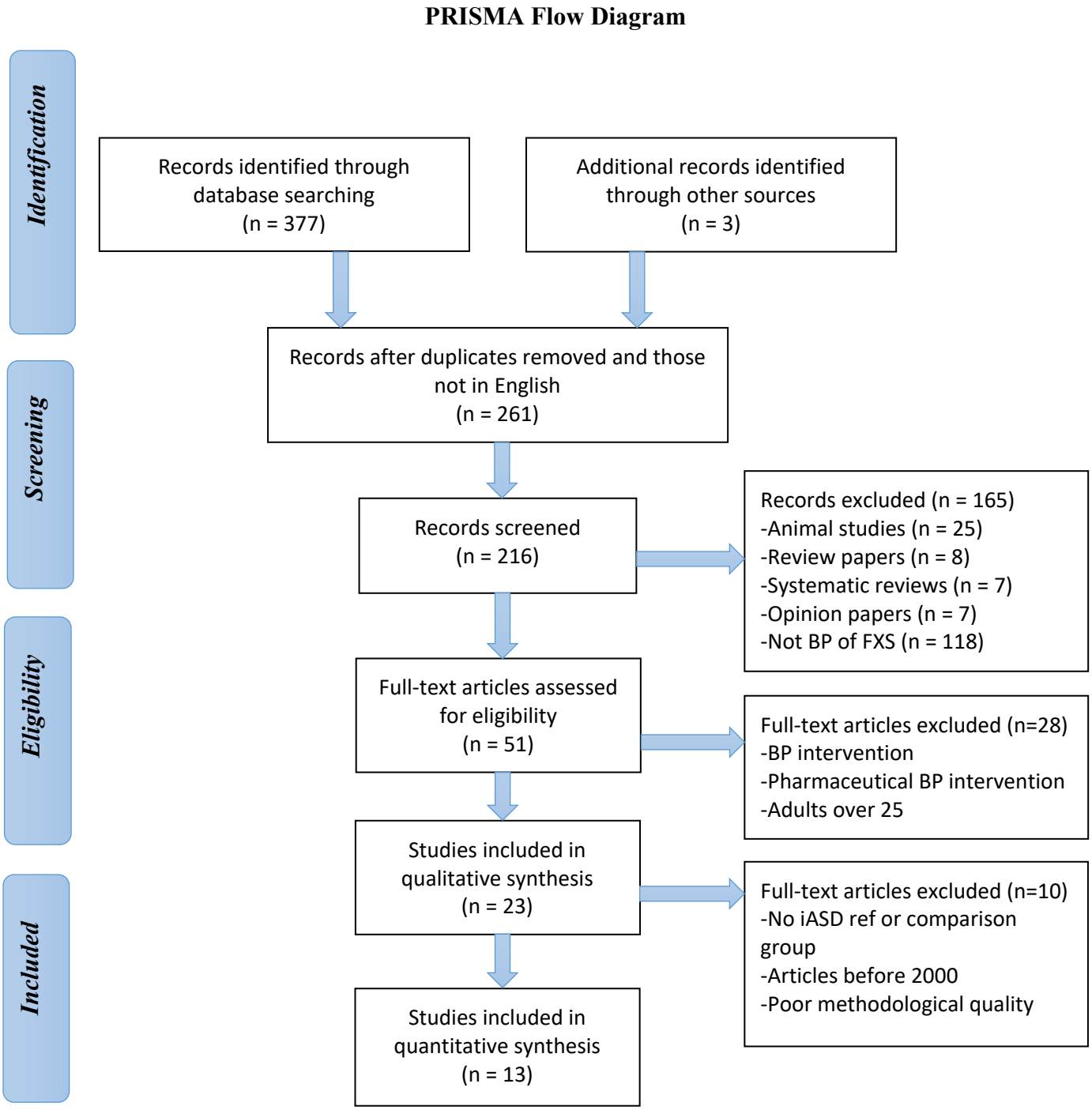


Figure 1 PRISMA flow-chart

The PRISMA flow-chart (Figure 1) demonstrates the filtering process of the 377 papers returned by the original search, 216 abstracts were retrieved after removal of duplicates, limiting to English

language and human studies. Following analysis of abstract, 51 papers were retrieved in full and further papers were retrieved from hand searches of these references.

Data extraction and scoring

Two independent assessors ratified all papers for quality using an adapted version of Cross and Hare's (2013) generic tool to review and score the quality of studies assessing BP (see Appendix B). In total, 23 papers were screened for methodological quality and specificity (see Appendix C).

An additional criterion was applied to exclude studies that didn't compare FXS to an ASD control or normative data for individuals with ASD e.g. ADOS or ADI-R, resulting in 13 studies for review. Papers that scored ≥ 9 were deemed to warrant inclusion.

Methodology

Papers for review (N=13) were then scored for methodological quality, summarised in Table 1.

Table 1: Summary of methodological quality of selected review papers.

Author/ Year/ Score	Study Aims	Control Group	Sample Size (age range)	Recruitment	Diagnosis	Methodology	Developmental Factors	Stats	Findings
1.Hall et al. 2010	Determine appropriateness of comorbid ASD diagnosis in boys and girls with FXS.	FXS (n=120) ND	N=120 (47F/73 M) AR=5-25	MC	SBT ADOS	S/VM	SA	DS, BC, WCY* OR	35% boys and 4.31% girls with FXS scored on the ASD category on SCQ and ADOS; poor agreement between measures. Significant differences in profile of ASD symptoms in FXS+ASD compared to norms for ASD. (FXS+ASD=High levels of social avoidance, repetitive behaviours & language, but less impaired than IA). IQ significantly negatively associated with SCQ.
12/14		**1**	**2**	**2**	**2**	**1**	**2**	**2**	
2. Hernandez Feinberg, Vaurio, Passanante, Thompson, & Kaufmann, 2009	Stability of FXS+ASD & cognitive ability over time	FXS + (n=24 at baseline) FXS (n=32 at baseline)	Time 1: N=56 AR=30-88 months Time 2: (n=44) Time 3: n=34)	SC	SBT ADI-R	S/VM	LS	DS, BC, WCY*	Diagnosis of FXS+ASD stable over time, with behaviours linked to peer relationships & socialisation adaptability. Reciprocal Social Interaction (Recs) only domain to distinguish FXS+ASD vs FXS at all time points. Increased severity in FXS and reduced severity in FXS+ASD over time meant that differentiation between the two was harder. FXS+ASD IQ scores were stable over time, but FXS Verbal IQ decreased over time.
12/14		1	2	1	2	2	2	2	
3.Kau et al., 2004	Social behaviour profile (SBP) of boys with FXS, and effect of age	FXS (n=41) FXS+ASD (n=14) DLD with ASD; (n 22) iASD (n=11)	N=88 AR=3-8	MC	GT SBT ADI-R	S/VM	AM (FXS / DLD + ASD)	DS, BC WCY*	FXS+ASD is a distinctive sub-phenotype in boys with FXS.
13/14		2	2	2	2	1	2	2	

4.Klusek, G. E. Martin & M. Losh (2014)	Whether boys and girls with FXS met criteria for ASD using ADOS/ADI	FXS (n=86) ND	N= 86 (35 F / 51M) AR M/SD	MC	NS ADO S/AD I-R	S/VM CI OM	SA	DS, WC Y* OR	43% sample met criteria for ASD using ADOS & ADI-R. ADOS/ADI-R agreed 76.5%. 56% caregiver reported diagnosis agreed with ADOS/ADI-R. ASD may be under diagnosed in clinical settings.
11/14		1	2	2	0	2	2	2	
5.Lee, Martin, Berry-Kravis, & Losh, 2016	BP change over development (mean 2.5 years) in boys & girls with FXS.	FXS (31 M; 34 F) ASD-O (19 M)	N=84 NS M/SD	MC	NS ADO S/AD I-R	S/VM	SA	DS, WC, BC Y*	ASD symptoms increased in FXS with age; social language impairment emerged as a potential core shared feature of FXS and ASD.
12/14		2	2	2	0	2	2	2	
6.Martin, Bush, Klusek, Patel, & Losh, 2018	language sample analysis of syndrome and sex-specific profiles	FXS+ASD n=61 FXS n=40 DS n=42 TD n=37 ASD n=29	N=209 NS M/SD	MC	GT ADO S	S/VM OM	SA	WC, BC Y* ES	Non-continent language and perseveration were characteristics of the pragmatic profiles of boys and girls with FXS+ASD and boys with ASD. Boys with ASD initiated turns less often and were more non-responsive than other groups; girls with FXS+ASD were more non-responsive than male counterparts.
14/14		2	2	2	2	2	2	2	
7.McDuffie, Thurman, Hagerman, & Abbeduto, 2015	Which symptoms of ASD differed in boys with FXS relative to same aged boys with ASD.	FXS n=57 ASD n=61	N= 118 AR=4-10	MC	GT ADI-R/ADOS GS	S/VM	AM	DS, BC Y* ES	Boys with FXS show significantly less impairment in social smiling than did age, severity and diagnostic boys with iASD. Severity matched boys with FXS showed more impairment in complex mannerisms than boys with iASD.
13/14		2	2	2	2	1	2	2	
8.Roberts et al., 2018	Social anxiety and FXS and association with ASD;	FXS n=59 (broken down to FXS and FXS+ASD for analysis) ASD n=18	N=77 AR=15-23	MC	GT ADO S/AD I-R GS	S/VM BCL	SA	DS,BC Y* ES	Salivary cortisol appears to be a marker of general disrupted arousal rather than specific indicator of social avoidance. Difference between FXS and FXS+ASD males; FXS had initial social avoidance; FXS+ASD has prolonged social avoidance.
14/14		2	2	2	2	2	2	2	
9.Rogers, Wehner, & Hagerman, 2001)	Symptoms of ASD and relationships between ASD symptoms and developmental	FXS (n=16) FXS+ASD (n=8) ASD (n=27) Other development	N=74 AR: 21–48 months	MC	GT ADI-R/ADOS.	S/VM:	Compared ‘en masse’	DS BC Y*	FXS group composed of 2 subgroups (FXS-O vs. FXS+ASD) prompting hypothesis for a genetic distinction.

	variables in young children with FXS	al delays (n=23)							
11/14		2	2	2	2	1	0	2	
10.Thurman, McDuffie, Hagerman, & Abbeduto, 2014)	Examine psychiatric symptoms in boys with fragile FXS using a parent report instrument.	FXS (n= 41) ASD (n=41)	N=82 AR=4.02-10.99	MC (2)	GT ADOS GS	S/VM:	AM (Sub-group n=30)	DS WR BC Y* ES	Symptoms of hyperactivity and general anxiety more frequent for FXS than ASD. Also positive association between social avoidance and general anxiety in FXS not found in ASD.
12/14		2	2	2	2	1	1	2	
11.Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015a	Compare profiles of ASD relative to age (CA), nonverbal IQ, and expressive vocabulary ability between FXS and iASD	FXS (n=12) FXS+ASD (n=41) ASD (n=39)	N= 92 AR=4-11	MC (2)	DPGT ADOS/ADI-R GS	S/VM:	AM NVIQM EVAM	DS BC Y* ES	Onset of ASD symptoms and developmental trajectories in males with FXS differ as a function of CA, nonverbal cognitive ability, and expressive vocabulary relative to males with iASD.
13/14		2	2	2	2	1	2	2	
12.Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015b	Evaluate the ability of males with FXS, ASD & typical development to learn new words by using cue	FXS (n = 32) ASD (n = 32) TD (n = 32)	N=172 AR=2.05 – 10.86	MC (2)	DPGT ADOS/ADI-R GS	S/VM OM (successful / unsuccessful search condition)	SA	DS BC WC Y* ES	*Successful search condition*: iASD performed similarly to FXS controlling for severity of ASD. *Unsuccessful search condition*: FXS performed significantly worse than iASD, controlling for severity of ASD.
14/14		2	2	2	2	2	2	2	
13. Wolff. Bodfish, Hazlett, Lightbody, Reiss, & Piven, 2012	Similarities and differences in behavioural expression of autism in FXS and iAut.	FXS (n=23) IA (n=38)	N=61 AR=3-5	MC	GT ADOS/ADI-R	S/VM	AM	DS BC Y* ES	Findings indicate phenotypical heterogeneity of autism in its unique presentation in FXS.
13/14		2	2	2	2	1	2	2	

Key for table

NS= Not specified.
Control group: FXS+ASD = Fragile X Syndrome and Autism Spectrum Disorder. ASD = Autism Spectrum Disorder. IA= Idiopathic Autism. DLD=Developmental Language Delay. TD = Typically developing. DS = Downs Syndrome. ND = normative data
Sample size: AR= Age range. M/SD = M/SD reported
Recruitment: SC-=Single clinic or diagnostic centre. MC= Main diagnostic clinic or multiple clinics / centres
Syndrome diagnosis / ASD diagnosis based on: GT=Genetic testing**.** DPGT = Document of proof for genetic testing. ADI-R = Autism Diagnostic Interview Revised (ADI-R). ADOS = Autism Diagnostic Observation Scales (ADOS) / ADOS 2**.** GS = Genetic screening (to rule out FXS)
Methodology: CI=Clinical interview**.** BCL=-Baseline cortisol level. OM=Other methodology**.** S/VM=Standardised/Validated measures
Developmental Factors: LS=Longitudinal study**.** AM=Age Matched Control**.** NVIQM=Non-verbal IQ matched**.** EVAM=Expressive vocabulary ability**.** SA=Accounted for by statistical analysis
Statistics: DS=Descriptive statistics/percentages. WC=Within syndrome comparative statistics. WR=Within syndrome correlations. BC=Comparative statistics between syndrome and genetically distinct control group. Y/N (*) = sig. diff. found from genetically distinct control / repeated measures Y/N ES = Effect size reported
OR = Odds ratio reported

Findings

All studies were rated as being of good methodological quality and so were reviewed in more detail. The findings of this review were grouped according to aspects of the behavioural phenotype, as well as IQ, with comparisons made between findings, reference to methodology and limitations highlighted.

Social Interaction

A diagnosis of ASD in FXS was relatively stable over time, with prominent behaviours linked to peer relationships, socialisation adaptability and social withdrawal behaviours mainly informing ASD diagnosis. Reciprocal Social Interaction was the only domain to distinguish FXS+ASD from FXS, being significantly more impaired, at all-time points (Hernandez et al. 2009).

Lee et al. (2016) indicated similarities on specific symptoms between ASD and FXS+ASD group at time point one, which are not demonstrated in FXS. Significant differences in facial expressions and social overtures, were found, with FXS less impaired than FXS+ASD, who were less impaired than ASD.

Contrary to previous research (e.g. Hatton et al., 2006; Hernandez et al., 2009) ASD symptoms in FXS increased with age. Social language impairment emerged as a potential core shared feature of FXS and ASD. The greatest overlap was that of reciprocal social communication of boys with FXS+ASD when compared with boys with iASD. Thurman et al. (2015a) found that social

affective symptoms of ASD were significantly fewer in boys with FXS compared to iASD at age four; severity of symptoms increased with age for the FXS group, consistent with Hall et al (2010) and Lee et al (2016). Symptom severity is not considered to be age related and so indicates a tangible change over time. A significant between group difference emerged with severity of social affect minimally affected by age, nonverbal IQ and expressive language ability in iASD contrasting with that for children with FXS; support for the finding was reported in Kau et al. (2004) and Lee et al. (2016).

Specific differences were highlighted by Hall et al. (2010) with both genders with FXS indicating lower impairment on communication and reciprocal social interaction items than the referenced ASD samples, as well as high levels of social avoidance, repetitive behaviour and language difficulties present in FXS+ASD, but not to the level of impairment noted in iASD.

Thurman et al. (2015a) found boys with FXS demonstrated less impairment than ASD CA matched participants in reciprocal interaction behaviours. FXS+ASD demonstrated less impairment than ASD in social smiling and showing and directing; and when matched for severity this was limited to social smiling. FXS demonstrated significantly less gesture use than ASD CA matched participants. Similarly, Wolfe et al. (2012) found those with FXS+ASD were significantly less severe than iASD on five ADOS measures of social behaviour. These findings were contrary to Rogers et al. (2001).

Social Communication

Thurman et al. (2015a) found that in verbal participants, social verbalisation and pronominal reversal was less impaired for FXS compared to CA matched, but not FXS+ASD. Thurman et al. (2015b) evaluated the ability of boys with, FXS, iASD and TD to learn new words based on a social emotional cue, as an indicator of ability to understand social communication. In the successful search condition, their findings indicated those with FXS are less impaired than those with ASD and not significantly different to TD children, consistent with findings of Wolff et al. (2012). This study did not use an FXS+ASD control but did statistically control for ASD severity, following which there was no significant difference between the groups. In the unsuccessful search condition, it was the FXS group who demonstrated significantly lower performance than the TD and iASD groups (after controlling for ASD severity). The differences between condition indicate the potential role of social avoidance in poor word learning.

Further exploration of pragmatic language, undertaken by Martin et al. (2018), found both genders with FXS+ASD had profiles matching boys with iASD. Boys with iASD initiated turns less often and were more non-responsive than other groups, whilst girls' non-responsiveness was less favourable than boys within the FXS+ASD group. Boys with FXS+ASD and iASD used more non-contingent language than boys with FXS, DS and TD, providing support for a continuum model of impairment. Whereas girls with FXS+ASD were more non-contingent than girls across all other groups, a difference that could be a specific BP for girls with FXS+ASD.

Roberts et al., (2018) utilised an adolescent and young adult male sample to study the behavioural and biological aspects of social anxiety in FXS, FXS+ASD and ASD. The FXS group had initial social avoidance and FXS+ASD group had prolonged social avoidance, in line with ASD BP. Salivary cortisol appeared to be a marker of general disrupted arousal rather than specific indicator of social avoidance. However, limitations included a smaller sample size of ASD, not matched on nonverbal IQ or expressive language. The study highlights the complexity in measuring cortisol, anxiety and ASD traits in FXS and conclusions echo existing literature (e.g. Hardiman & Bratt. 2016; Corbett et al., 2006; Spratt et al., 2012) that the relationship remains unclear.

Thurman et al. (2014) examined the profile of anxiety in boys with FXS compared to iASD. They reported a positive association between social avoidance and general anxiety in FXS, more so than in iASD when controlled for CA, nonverbal cognitive ability and or ASD symptoms. Conclusions from this study highlight that anxiety is a significant contributory factor in functioning for boys with FXS, a potential key difference in the development of phenotypes of FXS and iASD which would provide validity for the need for different target intervention between the two. Limitations included assessing psychiatric symptoms through parental report and narrowed focus on boys.

Restricted Interests/ Repetitive behaviour

Boys with FXS and FXS+ASD demonstrated significantly lower restrictive and repetitive behaviour severity scores than boys with iASD. (Kau et al., 2004; Lee et al., 2016). Kau et al (2004) established iASD showed greater impairment in restricted and repetitive behaviours at a later time point. Kau et al (2004) conclude that stereotypical behaviours (and communication

impairment) contribute more to the diagnosis of ASD in the FXS+ASD group. These results fit with the finding reported by Rogers et al (2001). They concluded that there is a distinctive SBP sub-phenotype in boys with FXS which may share pathophysiological mechanisms reported in iASD.

Similarly, Thurman et al (2015a) concluded that in restricted or repetitive interest items FXS were significantly less impaired than iASD for unusual preoccupation and compulsions and rituals than CA and FXS+ASD matched groups. Complex mannerisms were observed to be more impaired in boys with FXS than severity-matched group.

Rogers et al. (2001) found an FXS group presented with higher ASD traits than a DD group of young children (21-48 months). The iASD and FXS+ASD did not differ significantly on any of the variables. The FXS-O and iASD group differed significantly on all but the Repetitive behaviours and stereotyped patterns scale of the ADI-R. Within the FXS cohort two sub-groups emerged: FXS group (virtually identical performance to DD group) and an FXS+ASD group (virtually identical performance to iASD group). Consistent with the literature (e.g. Hall et al., 2010; Kau et al., 2004; Smith et al., 2012), Rogers et al. (2001) support the perspective that children with FXS+ASD present with an extension of the FXS BP rather than a distinct condition that sets them apart from children with FXS-O. They attempt to explain this by the young chronological and developmental age of the sample and the lack of a developmental trajectory methodology.

Wolff et al. (2012) used a similar aged sample (3-5 years) but found significantly less compulsive or ritualistic behaviour in boys with FXS+ASD than boys with iASD; contrary to McDuffie et al (2015) who used older children and found FXS were significantly more impaired than iASD in compulsive and ritualistic items of this ADI-R scale.

IQ & Adaptive Behaviour

There is already a consensus regarding the high frequency of ID in males and less frequent presence in females (e.g. Hagerman et al., 2009; Gallagher & Hallahan, 2012; Crawford et al., 2015). Hall et al. (2010) used a regression model to show IQ was significantly negatively associated with SCQ score in both girls and boys with FXS (Hall et al., 2010).

Increased severity in FXS and reduced severity in FXS+ASD over time (despite stability in diagnosis) means differentiation between the two presentations was harder. FXS+ASD IQ scores were stable over time, but FXS verbal IQ decreased over time (Hernandez et al., 2009). The latter outcome is a consequence of the decline in VIQ over time, relative to TD children (Hernandez et al., 2009). However, raw scores of VIQ are not reported.

Kau et al. (2004) suggest that FXS+ASD present increased problematic behaviour, reduced cognitive ability and adaptive behaviour. Specifically, the FXS+ASD had lower IQ scores, displayed greater impairment in aberrant and problematic behaviour, particularly regarding social avoidance/withdrawal with lower age equivalent scores of adaptive behaviours.

Lee et al. (2016) found over time girls demonstrated reduced rates of ASD symptoms relative to boys but not when controlling for structural language and mental age. They indicated that pragmatic language ability was a significant variable influencing ASD assessment outcome in individuals with FXS.

Methodology observations

Hernandez et al. (2009), Lee et al. (2016) and Thurman et al. (2015a) all utilised a longitudinal methodology. These studies highlight the value of a developmental perspective in FXS and limitations of using a single time point for comorbid ASD assessment and diagnosis in clinical practice. However, the reliance on the ADI-R by Hernandez et al. (2009) as the sole measure of ASD is a limitation, due to the disadvantages of sole reliance on parental report. Although the other studies utilised the ADOS in addition to the ADI-R, there is evidence of a lack of consensus between the measures (e.g. Hall et al., 2010; Klusek et al., 2014) which is not addressed. In addition, Lee et al. (2016) assessed the majority of participants at time 2 using ADOS module 3, therefore diagnosis was more reliant on expressive language, thus limiting generalisability to verbal children.

Hall et al. (2010), Kau et al. (2004), Klusek et al. (2014) and McDuffie et al (2015) used samples with a chronological age mean that was older than the early infant studies (e.g. Rogers et al., 2001; Wolff et al., 2012), although Hall et al. (2010) ranged to young adulthood (5-25 years). Hall et al. (2010) and Klusek et al. (2014) describe similar percentages of FXS+ASD. Both studies highlight limitations with parental and observational measures used. Wolfe et al (2012) acknowledge that

their use of the ADOS may have led to rater error and limited sample selection. However, only approximately half of the Hall et al. (2010) sample were assessed using ADI-R and ADOS, which limits the findings due to differences in diagnostic outcomes for these measures indicated by Klusek et al. (2014).

The findings of Thurman et al (2015a) are limited by the nature of the ADI-R reliance on parental report and lack of supported from a practitioner administered observational measure. They are also not generalisable to participants with IQ greater than 85. Thurman et al. (2015 b) had similar limitations to that of Thurman et al. (2015a); where individuals with ASD with higher IQ (>85) were excluded, as such, the findings are not representative of boys with iASD. Sample size was also fairly low for requirements of a regression model and the ADOS was not administered to the TD group to rule out any undiagnosed children, so effecting certainty that the differences across groups were due to presentation.

The majority of studies utilised solely male participants; however, gender differences were considered by Hall et al. (2010), Klusek et al. (2014) and Martin et al. (2018) who consistently reported ASD as more prevalent in boys than in girls with FXS (Hall et al., 2010, Lee et al., 2016, Klusek et al., 2014). This is also supported in the wider literature (e.g. Clifford et al., 2007); although Klusek et al (2014) reported that in clinical practice only 25% of both genders received a diagnosis which may reflect under diagnosis.

The generalisation of findings by Hall et al. (2010), Klusek et al. (2014) and Lee et al. (2016) are limited for girls to those with expressive language. The developmental trajectory implications

indicated by Hernandez et al. (2009), Lee et al. (2016), Thurman et al. (2015a) and Hall et al. (2010) suggest that findings should be interpreted with caution due to the difference between the mean and control sample ages. Martin et al. (2018) had similar limitations having been unable to recruit non-verbal mental aged matched participants, instead using statistical analysis. The latter study acknowledges that the examiner-child interactions does not portray an accurate assessment of ability in real-life situations, impacting on generalisability. Limitations of Kau et al (2004) include that the iASD group were significantly older than that of the FXS+ASD group, which considering the developmental trajectory of FXS+ASD, highlighted previously, is key. The FXS group were significantly more impaired on IQ scores than the DLD group.

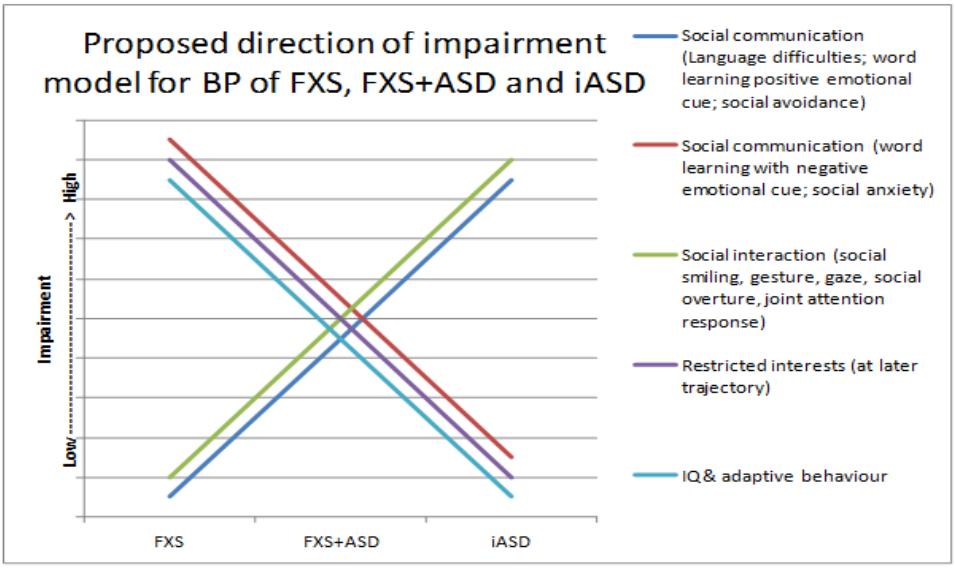
Discussion.

This systematic review examined N=13 papers of good methodological quality that reported on ASD traits in children and young people with FXS. The findings of these do not provide evidence that ASD traits in FXS represent a categorical distinct disorder within FXS that would achieve status within the DSM-V or ICD-10 as FXS+ASD as opposed to a comorbid ASD diagnosis. Instead, there appears to be a consensus that the distinction between ASD and FXS BP is based on variation in impairment. The current review considers a continuum model helpful for synthesising these findings to distinguish the direction of impairment on different BPs underpinning FXS, FXS+ASD and ASD; as well as a trajectory model to explain the development of BP over time, illustrating a similar developmental trajectory for FXS and FXS+ASD over time, with symptomology severity or impairment increasing with chronological age; whereas trajectory for ASD symptomology, although heterogeneous, is stable over time.

Proposed provisional behavioural phenotype continuum of FXS, FXS+ASD, ASD:

The papers reviewed provide varying amounts of evidence for the presence of a BP for FXS+ASD which differs from the BP for FXS and iASD. Figure 2 displays an illustration of the comparative level of impairment associated with BP in individuals who present with FXS, FXS+ASD or ASD. The distinction between FXS, FXS+ASD and ASD appears best explained on a continuum, with impairment associated with BP differing comparatively between the three presentations. A discussion of the evidence contributing to this illustration follows.

Figure 2: Behavioural phenotype demonstrated in FXS, FXS+ASD and iASD, with indication of the comparative direction of level of impairment where there is a consensus in the review



Synthesis of study outcomes highlights the significant contribution of social interaction characteristics that contribute to the differentiation in the distinct characteristics of FXS, FXS+ASD and ASD (eg. Hall et al., 2010; Hermandez et al. 2009; Lee et al., 2016; Wolff et al., 2012; Rogers et al., 2001). Ultimately, there is a consensus that reciprocal social interaction characteristics are more impaired for ASD than FXS+ASD, which are more impaired than FXS. However, there is a disparity in the direction of impairment for BPs that make up social communication, highlighted by the social element of language development and the distinction between social anxiety (e.g. Kau et al. 2004; Klusek et al., 2014; Thurman et al. 2010) and social avoidance (e.g. Roberts et al., 2018; Hall et al. 2010).

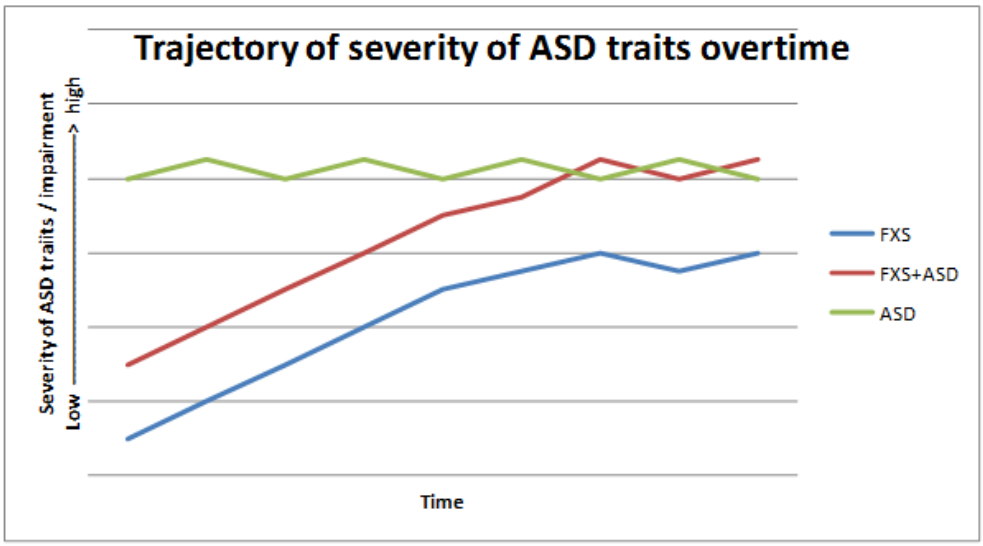
Social anxiety BP are significant in distinguishing between FXS, FXS+ASD and ASD, with the direction of impairment indicating FXS are more impaired than FXS+ASD who are more impaired

than ASD. Consideration of the BPs associated with language acquisition were fundamental to understanding this finding, with emotional cuing indicating the impairment in social communication (e.g. Thurman et al. 2015a; Thurman et al. 2015b). The difference in direction of impairment of social avoidance and social anxiety may be explained by the findings from cortisol studies that indicate disrupted arousal present in FXS, rather than pure social avoidance. Thus, suggesting a biological underpinning, regardless of social situation, which may be unresponsive to psychological or behavioural intervention.

Stereotypical behaviour is a significant BP for those with FXS+ASD, who present with more impairment than FXS alone (e.g. Rogers, 2001; Thurman et al., 2010; Lee et al., 2016). However, it appears to be a feature of the FXS BP that develops *later* on the developmental trajectory. Its absence in younger children with FXS may be key to a diagnosis of ASD not being provided (Kau, 2004). Consequently, it is proposed as a key item to track over time, but with consideration of the confounding variable of ID due to the association of increased repetitive behaviour and ID. Impairment in adaptive behaviours appears to correlate with impairment in IQ, which is more impaired in FXS than FXS+ASD, than ASD (e.g. Hernandez et al., 2009; Kau et al. 2004; Klusek et al., 2014)

Overall, synthesis of the reviewed studies suggests a developmental trajectory of impairment, indicating increased impairment over time for FXS (Rogers et al., 2001). The trajectory model is presented visually in Figure 3.

Figure 3: Illustration of the proposed trajectory of severity of ASD traits displayed in the BP of FXS, FXS+ASD and iASD over time



Review limitations

Ultimately, this systematic review was limited by the nature of evidence that it reviewed. Studies were limited to cross sectional and cohort studies, with 2 longitudinal studies. The review did not include intervention studies and consequently no randomised controlled trials (RCTs) were included. General limitations of the review included the heterogeneous nature of the studies; their participants; the BP being measured; their methodology; their assessment measures. The diverse nature of the studies meant that although odds ratio (OR) or effect size (ES) was reported in some studies, it was only appropriate to conduct a review of the studies and their data analysis, rather than conduct a meta-analysis of their ORs.

In addition to the potential limitation of over-reliance on parental report for assessment, parents of children with FXS provide the additional potential disadvantage of presenting with associated BP or symptoms associated with the syndrome. A significant limitation in the review studies is the weighting of male participants in studies, due to the associated lower IQ and increase prevalence of ID in males with FXS. Research into the BP of iASD traits in individuals with a diagnosis of FXS to pursue findings highlighted by Hall et al. (2010) regression analysis with regard to the role of IQ in ASD BP within FXS, would be more successful through analysis of female participants due to the reduced variability in cognitive ability.

The review was limited to BP underlying FXS and ASD presentations; however, there is also significant comorbidity with FXS and ADHD, which warrants exploring and the overlap with some BP of ASD may provide further insight into understanding FXS. As such, the review exclusion criteria also limited a much broader focus of potential underlying mechanisms involved in the development or cause of BP, with a narrower focus on the observable BP across FXS and iASD.

Research implications

This review has highlighted some testable hypotheses for future research, illustrated in Figure 3, including the following proposals: increase of repetitive and restrictive behaviour over time in FXS+ASD compared to FXS; increased impairment in reciprocal social interaction BP; and the specific pragmatic language deficits. Future research needs to address the limitations of the current review and associated studies while attempting to utilise female participants, with a longitudinal focus and minimise the reliance on parental reports of behaviour. A move towards longitudinal

studies is needed to test out the trajectory hypothesis arrived at by this review. Research examining the outcomes of interventions for symptoms present across FXS, FXS+ASD and iASD with a focus on outcomes for underlying biological symptoms, such as the role of cortisol, may be key to providing further distinction.

Clinical implications and conclusions

Rational for this review emerged following debate regarding the distinction between FXS and FXS+ASD and whether the latter was a distinct disorder. The current review has considered evidence for BPs to clarify this and proposed a trajectory model, as well as indicated direction of impairments for the distinctive BPs of FXS and FXS+ASD. As a consequence, the term *FXS+* is proposed as a descriptor for FXS+ASD, with the rationale that it highlights FXS+ASD as demonstrating more significant ASD traits and associated impairment. This hypothesis leads to the question of whether FXS and FXS+ are genetically distinct, with alleles present in the latter group that determine the development of ASD characteristics.

There is an undeniable under-diagnosis in clinical practice of FXS+ (Klusek et al., 2014) and BP that mirror ASD can go undiagnosed. The status quo reinforces demand for clinical services to accept cases based on symptomatology, need and QoL as opposed to diagnosis. While clinical service provision continues to be organised based on the categorical system of diagnosis supported by DSM-IV & ICD-10, FXS may not receive a service that could improve QoL. The current review highlighted the significance of impairment in reciprocal social interaction, restrictive and repetitive behaviours, and social language difficulties for individuals with FXS+. Specifically,

indicating the biological underpinning of social avoidance in FXS with indications for the clinical management of the BP.

The developmental trajectory of FXS appears the most significant factor for consideration in clinical practice and future research as this review concludes the behavioural phenotype of FXS changes significantly with CA in contrast to iASD BP, which on the whole remains fairly consistent throughout development. Of particular relevance is the contribution of reciprocal social interaction, which presents across developmental stages and restrictive and repetitive behaviours that increase in impairment over time. Current service provision and capacity makes diagnosis for ASD likely to result from assessment at one time point in clinical practice, this may exclude for children with FXS from relevant services given the trajectory of FXS+ symptomology.

It is likely that clinical guidelines documenting an appropriate diagnostic pathway and associated interventions for FXS+ would increase the percentage of diagnoses and subsequent relevant service access for this group. Considering the high prevalence of ASD traits, ID and psychiatric symptoms in individuals with FXS, clinical guidelines targeting these presentations would benefit from providing reference to people with FXS and associated assessment and intervention guidelines. However, the development of such interventions relies on further clarification of the evidence base within the literature on FXS.

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A comparison of Behavioural Phenotypes of Autism Spectrum Disorder traits in boys and girls with Fragile X Syndrome.

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(See Appendix A)

Abstract

Background: Prevalence of Autism Spectrum Disorder (ASD) traits in individuals with Fragile X Syndrome (FXS) is significant, with boys displaying more severely impaired behavioural phenotypes (BP) than girls and a higher incidence of intellectual disability (ID). Distinguishing differences in the BP of girls and boys may provide insight into the relationship between FXS and ASD.

Methods: Parents of children with FXS (n=48) completed measures via an online platform advertised by the FXS society. Data was collected for 39 boys and 9 girls with FXS using the Social Responsiveness Scale – second edition; the Repetitive Behavioural Questionnaire 2 and the Wessex Questionnaire. Statistical analysis using Mann-Whitney U test, Kruskal-Wallis H test and partial correlation analysis was conducted.

Results: Severity of ASD traits in girls compared to boys with FXS did not differ significantly; the BP of FXS-O was significantly different to FXS+ASD; and ASD traits were not correlated with ID, irrespective of gender.

Discussion*:* The incidence of ASD traits in FXS increased with age, supporting a trajectory model for development of ASD BPs. The lack of distinction in ASD traits between boys and girls may reflect the BPs assessed by the measures, with psychiatric, language and executive functioning more difficult to assess via parental report measures. Limitations include the lack of clinician administered behavioural observational measures or assessment.

Introduction

Genetic testing has enabled accurate diagnosis of Fragile X Syndrome (FXS), which affects approximately 1 in 4,000 males and 1 in 6,000 females (Boyle and Kaufmann, 2010). FXS has low homogenous behavioural properties, often perplexed by a range of Autism spectrum Disorder (ASD) qualities; as well as variable presence of phenotypes including attention deficits, hyperactivity/impulsivity, hyperarousal, anxiety and self-injurious behaviour (Boyle & Kaufmann, 2010). Cognitive impairment is common, with FXS being the most commonly known genetic cause of intellectual disability (ID; Crawford, Acuña, & Sherman, 2001).

FXS results from a cytosine-guanine-guanine (CGG) expansion on the X chromosome (Xq27.3), creating a direct correlation in the number of CGG repeats in the sequence and the severity of the phenotype in terms of physique, intellect and behaviour. The expansion typically leads to a decrease or absence of the fragile X mental retardation protein (FMRP), which is the protein produced by the FMR1 gene. Pre-mutation individuals or carriers have expansions in the region of 60 – 200 repeats, which do not significantly affect the transcription of FMRP; whereas expansions above 200 CGG repeats lead to full mutation (O'Brien, 2006).

In contrast, the cause of ASD is largely idiopathic. An exception being FXS, accounting for approximately 5% of cases, which is the most common genetic cause of ASD (Budimirovic and Kaufmann, 2011). With prevalence rates estimated at 1 in 100 UK (Baird et al., 2006) and 1 to 68 in the USA (Baio, 2014), diagnosis of idiopathic ASD (iASD) is not as specific as FXS. Diagnosis of iASD has historically been based on the presence, observation and/or report of behavioural

criteria, which include difficulty in; reciprocal social interaction (RI); and the presence of restrictive and repetitive stereotyped behaviours (RRB; American Psychiatric Association; APA, 2013).

In the UK, the diagnosis of iASD is made using the Diagnostic Statistics Manual (DSM-V; APA, 2013) or International Classification of Diseases version 10 (ICD10; World Health Organization, 1992) criteria. Gold standard assessment requires the use of standardised measures that include; the Autism Diagnostic Interview – Revised (ADI-R; Lord et al. 1994); the Autism Diagnosis Observation Schedule (ADOS; Lord et al., 1989; Lord et al, 2000); or the Diagnostic Instrument for Social and Communication Disorders (DISCO; Wing, Leekam, Libby, Gould, & Larcombe, 2002). The requirements for diagnosis outlined in the DSM-V and the psychometric properties of the ADOS and ADI-R, which were not specifically designed, normed or calibrated for FXS may lack specificity and consistency in diagnosing syndrome variances.

Prevalence rates of FXS individuals proposed to have comorbid ASD fluctuate between 21 to 51% (Moss & Howlin, 2009), 22 to 33% (Zafeiriou, Ververi, Dafoulis, Kalyva, & Vargiami, 2013) and 30% (male only), with mixed sex rates at 22% (Richards, Jones, Groves, Moss, & Oliver, 2015). In a sample of 63 boys with FXS (aged 2-19), 68% met the criteria for ASD using the ADOS, ADI-R or DSM-IV. However, only 24% met the diagnostic criteria with a consensus on all three (Harris et al., 2008). This may indicate the inconsistencies of applying dichotomously defined diagnoses, to behaviours and symptoms that vary throughout a developmental trajectory (Hall et al., 2010). Although the phenotypical behaviour observed in FXS and iASD are similar (Hagerman et al. 1986), recent literature proposes that FXS presents its own unique behavioural phenotype

(BP; BPs for plural) that differs from that observed in iASD (Clifford et al. 2007; Harris et al. 2008; Moss & Howlin, 2009; McDuffie, Thurman, Hagerman, & Abbeduto, 2015).

The aetiology of ASD continues to be explored: Feinstein and Reiss (1998) proposed that ASD symptomology in FXS was underpinned by a specific genetic allele; while Baron-Cohen, Nikmeyer and Belmonte (2005) and Aueyung et al. (2009) are exploring the role of foetal testosterone and the links to autism traits in males and females. Advances in neuroimaging indicate structural and functional brain differences between individuals with FXS and individuals with iASD. These differences potentially arise from different neural substrates and reflect different underlying psychological impairments (Gallagher and Hallahan, 2012).

Early infant studies (Rogers et al, 2001; Wolff et al, 2017) and studies using older children (Hall et al., 2010, Kau et al., 2004, Klusek et al., 2014; McDuffie et al., 2015) support the findings of longitudinal studies (Hernandez et al., 2009; Lee et al. 2016) that the trajectory of FXS-O, FXS+ASD and iASD are different. Thomas, Daffin & Hare (in submission) propose a trajectory model of FXS and FXS+ with symptoms of ASD in FXS increasing with age, which differs from iASD BP which is generally stable over time. Specifically, restricted and repetitive interests appear to emerge at a later chronological age in FXS (Lee et al. 2016), which may contribute to under diagnosis of ASD in younger children with FXS. Hernandez et al. (2009) highlight the contribution of peer relationships, adaptability in socialisation, and s*ocial withdrawal,* to ASD diagnosis. They illustrate that increased problems with Reciprocal Social Interaction differentiate FXS from FXS+ASD, the latter experiencing elevated difficulties. Hall, Lightbody, Hirt, Rezvani, & Reiss (2010), found a positive correlation between ASD traits and IQ using the Social Communication

Questionnaire (SCQ), supporting findings that ASD traits increased with level of intellectual impairment (Hessl et al., 2009).

Gender differences

Females with FXS tend to have reduced levels of FMRP, but not a complete absence. This typically results in a less severe physical and less cognitively impaired BP, although in some cases presentation can be equivocal to males (Gallagher & Hallahan. 2012). Cognitive deficits are less common (approximately 25% have ID, (IQ < 70) amongst females with FXS (Hagerman et al., 1992) but when an ID is present it tends to effect executive functioning (Riddle et al., 1997) with girls presenting with significantly more executive functioning problems than boys (Rinehart, Cornish & Tonge, 2010; Klusek, Martin, & Losh, 2014).

Research into girls with FXS is not as substantive as that with boys, but it does highlight that a substantial number of girls present with symptoms related to inattention that cannot be explained solely by low cognitive ability (Mazzocco et al. 1998). Lee et al. (2016) concluded that girls displayed lower levels of ASD symptoms, but not when structural language and mental age are controlled for. Social language skills with interactions with others, such as eye contact, facial expressions and verbal communication were a significant variable influencing ASD assessment outcome in FXS (Lee et al., 2016). In social communication presentation, gender differences were noted by Martin et al. (2018) with girls being more non-responsive than boys within the FXS+ASD group. Boys with FXS+ASD used more non-contingent language than boys with FXS only and typically developing children but not boys with iASD only. Girls with FXS+ASD were more non-contingent than girls across all other groups.

Gender differences in psychiatric presentation are noted by Rinehart et al. (2010), who highlight that post puberty females are more likely to experience mental health problems and consequently require appropriate psychiatric and psychological psychoeducation and assessment regarding this. Psychoeducation is recommended as the initial treatment intervention for children and families with neurodevelopmental disorders. Given that the information provided is predominantly based on studies with male participants (Rhinehart et al., 2010) the validity and generalisability of the psycho-education for girls with FXS must be questioned.

Ranging between 50 to 77% of females will present with a BP of psychological difficulties associated with depression (Roberts et al., 2009) as well as observable behaviours relating to specific phobias, social anxiety, self-injurious behaviour, impulsivity, shyness, poor eye contact, hyperactivity and inappropriate affect (Reiss, Hagerman, Vinogradov, Abrams & King, 1988; Hessl et al., 2008). Similar to these findings, Hull & Hagerman (1993) compared females with FXS to their female siblings without FXS and characterised specific behavioural phenotypes that presented in the siblings with FXS. These included: attention difficulties and hyperactivity; impulsivity; poor eye contact; hand flapping and biting. Furthermore, high rates of emotional regulation difficulties, depression, social anxiety and agoraphobia have also been reported in female carriers of FXS (Hull & Hagerman, 1993; Franke et al., 1998; Hessl et al., 2008; Roberts et al., 2009; Bourgeois et al., 2009).

ASD traits appear more prevalent in boys than in girls with FXS (Clifford et al., 2007; Hall et al., 2010, Lee et al., 2016, Klusek et al., 2014). Klusek et al (2014) noted that in clinical practice this

fails to translate to a diagnosis, with only 25% of both genders receiving a diagnosis. Equally Bailey et al (1993) demonstrated that 5% of 40 girls with a diagnosis of ASD screened positive for FXS without prior knowledge that they may have the syndrome. Highlighting the potential for misdiagnosis across the two categories.

Research question

Research in to BPs that exist across disorders such as FXS and iASD could prove significant in elucidating gene behaviour relationships (Hall et al., 2010; Martin, Bush, Klusek, Patel, & Losh, 2018). More research is required to fully delineate the BP of ASD traits present in FXS and detailed characterisation of shared phenotypes is necessary to understand why not all ASD BPs present similarly across individuals with FXS (Lee et al., 2016). Additional research is required to explore the proposed trajectory model of FXS and FXS+ (Thomas, Daffin & Hare; in submission), which hypotheses that FXS+ASD is not a distinct phenotype from FXS-O but that symptoms of ASD in FXS increase with age. Specifically, research into females with FXS is limited in comparison to that of males. Comparison of males and females with a diagnosis of FXS is hypothesised to aid greater understanding of the range of BP of ASD traits present across individuals with FXS. This has important implications for the development of specific evidence-based interventions and appropriate support for individuals and their families.

The principle aim of the current study is to examine differences in the BP of boys and girls with FXS. Comparison of those who have a diagnosis of FXS only (FXS-O) will be compared to those participants who have a comorbid diagnosis of FXS and ASD (FXS+ASD).

Hypothesis 1: The severity of ASD traits in girls with FXS will be significantly lower from that observed in boys with FXS

Hypothesis 2: The behavioural phenotype of participants with FXS-O will not differ significantly from the behavioural phenotype of those with comorbid diagnosis of FXS+ASD.

Hypothesis 3: Both social communication difficulties and restrictive and repetitive behaviours will be positively associated with intellectual disability irrespective of gender.

Method

This study was undertaken in collaboration with UK Fragile X Society. It was the second in a series of studies of FXS in this collaboration. Consequently, some methods and design were matched with the previous study in order to pool data to reduce the demand on participating families of children with FXS.

Participants

Participants were recruited via the FXS Society's database and its associated social media outlets such as Facebook and Twitter. CEREBRA (Welsh charity for families of children with brain injuries) were also provided information on the study and invited to share with their members to participate. A primary inclusion criterion was being a parent of a child or children, under the age of 16 years, with a genetically confirmed diagnosis of FXS. Participant data from the first of the studies in this series (Daffin, Thomas, Hardiman & Hare; in submission) and additional data from participants in this study were combined. In total 48 participants completed the study. All 48 were recruited from FXS Society. No participants were recruited from CEREBRA. Completed data was returned on 39 boys and 9 girls with FXS. There was a relatively high rate of partial responders (n=43), whose data could not be analysed. Table 1 provides gender, age range and diagnosis information for children represented in the data.

Table 1: Gender, age range and diagnosis category of children represented in the data.

CLINICAL GROUP												
FXS+ASD (N=23)						*FXS-O (N=25)*					
GIRLS (N=3)			BOYS (N=20)			GIRLS (N=6)			BOYS (N=19)		
0-5	6-10	11-15	0-5	6-10	11-15	0-5	6-10	11-15	0-5	6-10	11-15
N=0	N=2	N=1	N=1	N=9	N=10	N=0	N=2	N=4	N=9	N=6	N=4

Key: FXS+ASD group contains participants with dual diagnosis of ASD and FXS. FXS-O group contains participants with FXS diagnosis only. Diagnosis for FXS was based on the affirmative parental response for the question "Does your child have genetic testing confirmation of FXS?". Diagnosis for ASD was based on the affirmative parental response for the question "Does your child have a diagnosis of an Autism Spectrum Condition?". 0-5, 6-10, 11-15 = age groups in years. N = number of participants in each category.

Measures

Measures completed by participants included the; *Social Responsiveness Scale – second edition (SRS-2*; Constantino and Gruber, 2012); the *Repetitive Behavioural Questionnaire 2* (*RBQ-2*; Leekam et al., 2007) and the *Wessex Questionnaire* (Kushlick, Blunden, & Cox, 1973). Table 2 documents the subscales comprised within each measure.

Table 2. Subscales contained with the SRS-2, RBQ-2 and Wessex Questionnaire

SRS-2 subscales	*RBQ-2 subscales*	*Wessex subscales*
Social awareness	Repetitive Motor Movements.	Continence
Social cognition	Rigidity/Adherence to Routine	Mobility
Social communication	Preoccupation with Restricted Patterns of Interest	Self-help skills
Social motivation	Unusual Sensory Interest	Speech and literacy
Restricted interests & repetitive behaviour		

The *SRS-2* is 65-item rating scale measuring deficits in social behaviour. The *School-Age form* takes approximately 15 minutes to complete by a parent or teacher who knows the child. It measures the severity of ASD symptoms presented by the child in their natural environment. It was developed for use with children 4 – 18 years of age and focussed on the child's level of social impairments. It assesses social awareness, social anxiety/avoidance, social information processing, capacity for reciprocal social communication, and autistic preoccupations and traits. Items are scored on a 4-point scale, ranging from *not true* = 1, *sometimes true* = 2, *often true* = 3, to almost *always true* = 4. *Scoring* the *SRS-2* can be done by hand or computer programme with results reported as *T*-scores (*M* = 50, *SD* = 10) for five treatment subscales: *receptive, cognitive, expressive, motivational* and *preoccupation.* When *interpreting* the *SRS-2, total score* is the most reliable measure of social deficits related to ASD. *T*-scores of 76 or higher are considered severe, suggesting that an individual has clinically significant deficits in social functioning; T-scores between 66 and 75 are considered moderate and indicative of some clinically significant social deficits; *T*-scores between 60 and 65 reflect mild difficulties in social behaviour; with T-scores of <60 reflecting that an individual probably does not have social difficulties indicative of an ASD diagnosis.

The *SRS-2* provides a total score and subscale scores; the total score is the most reliable indicator of social deficits related to ASD. The authors of the measure have reported strong *internal consistency* across gender, age and clinical subgroups within the clinical sample. The authors clinical sample yielded a total reliability coefficient of .95, evidencing strong consistency across items. The authors *Predictive validity* analysis of their sample resulted in a sensitivity value of .92,

suggesting that the scale identifies 92% of individuals accurately, and specificity value of .92, indicating that 92% of individuals without ASD will not be identified by the *SRS-2* as having the disorder. Thus, the SRS-2 does well in identifying those with and without characteristics of ASD. The authors described how the SRS-2 model aligns with the symptom criteria proposed for the *DSM-5* (Constantino & Gruber, 2012).

The *RBQ-2* is a twenty item, four-factor measure developed to measure unusual sensory interests, repetitive motor movements, rigidity/adherence to routine and preoccupations with restricted patterns of interests, which closely resemble the repetitive behaviour subtypes outlined by the ICD-10 (WHO, 1992). The RBQ-2 is directly derived from a standardised clinical interview tool, the DISCO (Wing et al., 2002) which has good inter-rater reliability and discriminant validity (Leekam et al. 2002; Nygren et al., 2009; Maljaars et al., 2012) as well as strong agreement with outputs from ADI-R (Nygren et al., 2009) and ADOS (Maljaars et al., 2012) which are validated and widely accepted clinical instruments. The *RBQ-2* was developed using two geographical subsamples and reports good internal consistency (alpha = 0.85), inter-item validity, and across samples reliability and validity. The coefficient alpha statistics for each of the four sub-scales (repetitive movements alpha = 0.80; rigidity alpha = 0.75; preoccupations alpha = 0.72; and sensory interest alpha = 0.66) suggest acceptable internal consistency. Originally validated for use with 2-year-olds, the RBQ-2 has gained acceptance as an appropriate measure for use with children and adolescents with ASD with good internal consistency across the whole scale (a=.86) and for both RSMB (a=.79) and IS (a=.83; Lidstone et al. 2014). Satisfactory endorsement of items and good internal consistency support the construct validity of the RBQ-2 in children and adolescents

(Barrett et al., 2015). Due to similar findings across studies, inclusion for the target population for the current study was deemed appropriate.

The *RBQ-2* is categorised into four different factors which reflect different subtypes of restrictive and repetitive behaviours such as Repetitive Motor Movements and Unusual Sensory Activities (see Table 3). Ratings are based on recent behaviour (observed in the last month). A total score (mean score 1.00 to 3.00) is calculated by adding the points given for each item in the measure and dividing by the number of questions answered.

Table 3. RBQ-2 sections, items and scoring response

Factor	*Item Numbers*	*Score and response*
Repetitive Motor Movements	2,3,4,5, 6	(1) never/rarely (2) once/twice a day (3) 15+ a day
Rigidity/Adherence to Routine	13, 14, 15, 16, 17,18, 19	(1) never/rarely (2) Mild/occasional (3) Marked/notable
Preoccupation with Restricted Patterns of Interest	7, 8, 10, 11, 12, 17	(1) never/rarely (2) Mild/occasional (3) Marked/notable
Unusual Sensory Interest	8, 9, 10, 18	(1) never/rarely (2) Mild/occasional (3) Marked/notable
Additional question	20	(1) Range of different and flexible self-chosen activities (2) Some varied and flexible interests but commonly chooses the same activities (3) Almost always chooses from a restricted range of repetitive

Key: *RBQ-2: Repetitive Behavioural Questionnaire 2; item response scoring options.*

The *Wessex Questionnaire* (Kushlick et al., 1973) is used to assess several dimensions of ability in children and adults with ID. Comprised of five subscales, with a total of 15 items: *continence* (4 items)*; self-help skills* (3 items)*; mobility* (2 items)*; speech* (1 items); *literacy* (3 items); *vision and hearing* (2 items); Items are scored on a scale from 1 to 3, higher scores indicating a greater level of ability. It has good efficacy for use in large scale questionnaire studies with good inter-rater reliability at both sub-scale and item level for adults and children (Richards, Oliver, Nelson, & Moss, 2012). A score of between 5 and 9 can be indicative of moderate to severe ID (Bell et al., 2018).

An online data collection platform was developed through Qualtrics (see appendix D) to providing a single point of access to these measures. Demographical and questions exploring health and support needs, based on work by Bromley, Hare, Davison and Emerson (2004), were additional items added to platform. Paper copies of the questionnaires were available on request. The online link was distributed via the Fragile X society and available for twelve weeks. Participants received: an information sheet outlining the research and its aims (Appendix E); information outlining access to appropriate support and guidance available from the FXS society should any distress arise (Appendix F); and a consent form for their data to be included in the study (Appendix G).

Ethics

The proposed project received ethical approval to the Cardiff University School of Psychology Research (Appendix H) and also approved by the Fragile X Society (FXS-UK) research board.

Results

Data Analysis Plan

Descriptive statistics were calculated to establish the means, standard deviation and ranges of all measures. When assessed for normality through analysis of histograms, box plots and formal tests of normality (Kolmogorov-Smirnov), significant skewness and kurtosis were apparent in the distribution for several key variables. Due to the unequal sample sizes and assumptions of normality not being met, as assessed by the Kolmogorov-Smirnov tests ($p<.05$), non-parametric analysis was recommended (Tabachnick & Fidell, 2014).

Kruskal-Wallis H tests and Mann-Whitney U tests were employed to test for between-group differences on SRS-2 and RBQ scores between gender and clinical groups. For hypothesis two, participants were put into 1 of 4 groups relating to Gender (Male / Female) by diagnosis (FXS-O / FXS+ASD). This led to the creation of four groups; Male FXS+ASD, Female FXS+ASD, Male FXS and Female FXS. Finally, hypothesis testing involved exploring associations between IQ and ASD symptomology using Spearman's Rank Order Correlations whilst controlling for gender.

Descriptive Data

The two samples (FXS and FXS+ASD) were matched group-wise on IQ scores. The male: female ratio was 19:6 in the FXS group and 20:3 in the FXS+ASD group. There were no significant group

differences for IQ scores (FXS group *Mdn*=36, IQR=6; FXS+ASD *Mdn*=35, IQR=6; U=265, z=-.465, *p*=.642).

Age and Diagnosis

Regarding age groups of participants and clinical diagnosis, a chi-squared (χ^2) test of association revealed that there was a significant different between age groups of participants and associated diagnosis $\chi^2 = 7.27$, $p = .026$. As shown in Figure 1, there were significantly more 6-10 years olds with FXS+ASD and significantly more 11-15-year olds with FXS+ASD compared to FXS only. However, there was significantly more 0-5-year olds with FXS than FXS+ASD.

Figure 1. Age groups of participants by Diagnosis

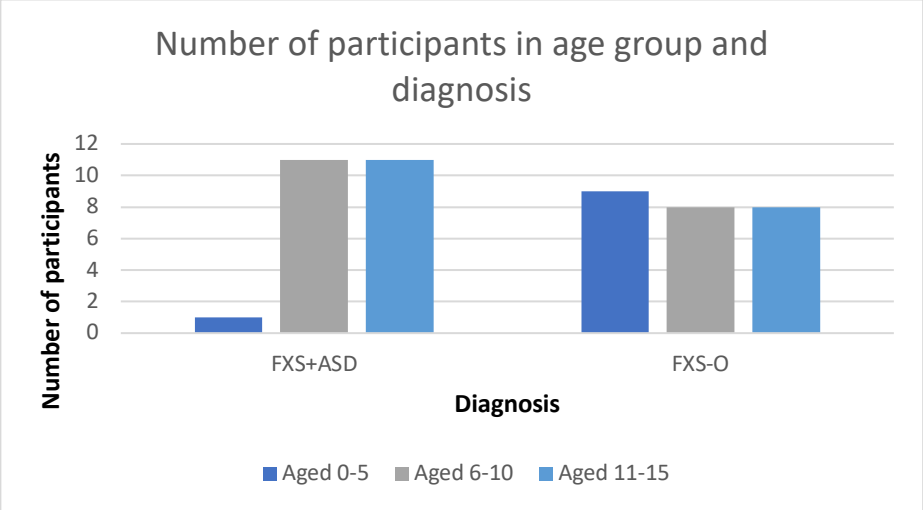


Table 4 provides means, standard deviations and ranges of measures for all participants and participant groups. Between-group differences on all measures are presented using Mann-Whitney U tests.

Of note, the FXS+ASD group showed significant higher scores (p<.008) on the *SRS-2 Total score* than the FXS-O group; there was also a between group significant difference (p=.001) on *SRS-2 motivation subscale;* and a significant difference (p=.016) on the *SRS-2 repetition subscale.*

On the *RBQ-2,* participants in the FXS+ASD group scored significantly higher than the participants in the FXS-O on the *Preoccupation with Patterns* subscale (p=.005*)* and the *Rigidity /Adherence to routine (p=.003)* subscale. No other significant differences between the FXS+ASD and FXS-O groups were found on the measures using Mann-Whitney U tests.

Severity across groups

According to severity ratings suggested for the SRS-2, 72% of the FXS group had severe levels of social difficulties. In the FXS+ASD group, 95% of participants had severe levels of ASD symptomology. This shows that the SRS is accurately measuring social difficulties integral to ASD.

Z-scores were calculated to highlight the profiles for the *RBQ-2, SRS-2*, and *Wessex* subscales. Figure 2 shows the *z-scores* of the girls in the study plotted against the *z-scores* of the boys acting

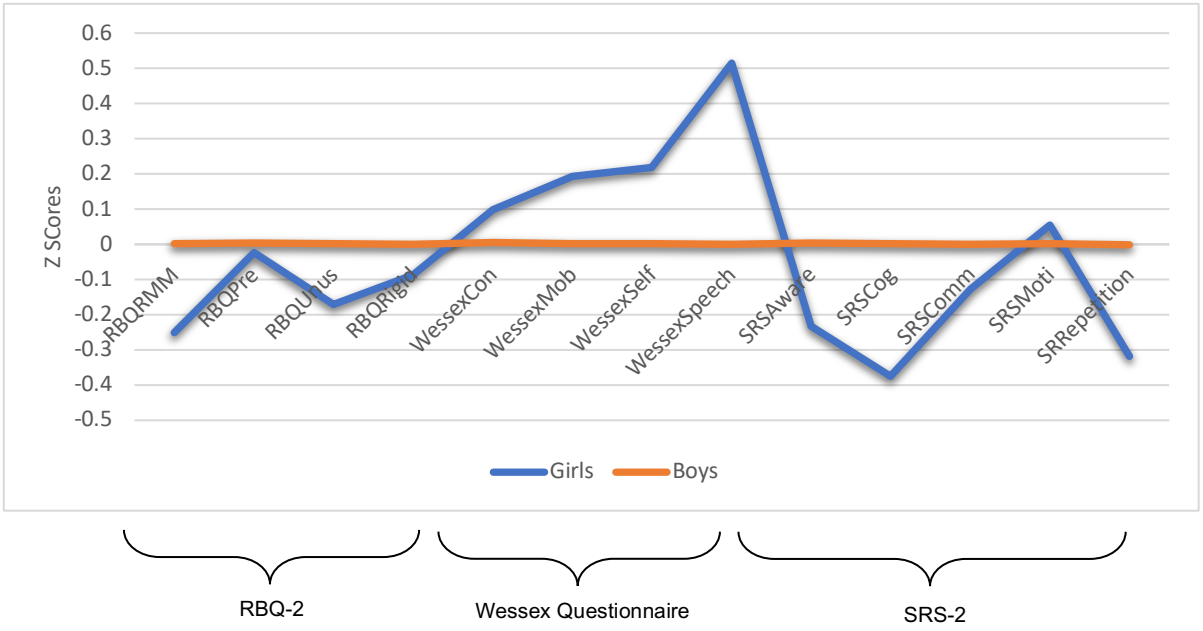
as control in order to provide a visual representation of the contrast in gender scores for the subscales.

Table 4. Descriptive statistics for all measures for sample (n=48)

Measure *(Subscales)*	Entire Sample (N = 48) Mean (SD) Range	FXS+ASD (N =23) Mean (SD) Range	FXS-O (N = 25) Mean (SD) Range	U	p-value
SRS-2 Total	151.16 (20.74) 113-212	158.26 (17.35) 121-188	144.64 (21.78) 113-212	159.00	**.008**
SRS-2 Motivation	27.29 (4.51) 17-38	28.30 (4.02) 20-38	23.48 (5.20) 17-38	115.00	**< .001**
SRS-2 Communication	41.14 (5.95) 31-57	40.22 (5.1) 32-51	42 (6.63) 31-57	209.00	.104
SRS-2 Cognition	28.41 (5.11) 18-42	27.96 (4.14) 19-35	28.84 (5.93) 18-42	233.00	.263
SRS-2 Awareness	19.77 (3.37) 11-31	18.96 (2.79) 11-23	20.52 (3.73) 15-31	282.50	.917
SRS-2 Repetition	34.54 (7.2) 16-51	36 (6.42) 23-46	33.2 (7.75) 16-51	171.50	**.016**
RBQ-2 Total	1.86 (.55) 1.05-3.50	2.00 (.53)1.05-3.15	1.72 (.55)1.10-3.50	195.00	.056
RBQ-2 Repetitive Motor Movements	1.88 (.69)1.00 – 3.80	1.96 (.64)1.00-3.20	1.82 (.74)1.00-3.80	235.00	.273
RBQ-2 Preoccupation with Patterns	1.10 (.38).57-2.00	1.25 (.36).57-2.00	.95 (.34).57-1.86	151.50	**.005**
RBQ-2 Unusual sensory interests	1.21 (.50) .75-2.25	1.30 (.61).75-2.25	1.13 (.38).75-2.00	263.00	.602
RBQ-2 Rigidity /Adherence to routine	2.14 (.68)1.14-3.71	2.43 (.67)1.00-3.20	1.87 (.58)1.14-3.71	145.50	**.003**
Wessex Total	34.58 (8.11) 21-51	35.34 (8.05) 21-51	33.88 (8.26) 21-47	265.00	.642
Wessex Continence	9.04 (3.01) 4-12	9.34 (3.14) 4-12	8.76 (2.93) 4-12	260.50	.567
Wessex Mobility	6.66 (2.43) 3-9	6.78 (2.61) 3-9	6.56 (2.31) 3-9	251.50	.441
Wessex Self-Help	9.52 (3.33) 5-15	9.78 (3.52) 5-15	9.28 (3.2) 5-15	260.00	.566
Wessex Speech	9.22 (3.34) 4-17	9.3 (3.28) 4-17	9.16 (3.46) 4-17	280.00	.876

Key: mean, standard deviation SRS-2: Social Responsiveness Scale – second edition total and subscale; RBQ-2: Repetitive Behavioural Questionnaire 2 total and subscale.

Figure 2. Cognitive profiles of girls plotted against the profile of boys as control.



The visual representation of Figure 2 adapted from Iqbal et al., (2009) highlights that girls have a higher cognitive ability, or reduced likelihood of an ID, than boys across the subscales on the Wessex measure. Girls in this sample show greater frequency of ASD traits across both SRS-2 and RBQ-2 measures with z-scores highlighting greater frequency of restrictive and repetitive behaviours and social interaction difficulties. Although z-scores for the girls indicate a greater score on SRS-2 social motivation subscale, the boys although less motivated, perform better at social interaction.

Inferential testing

Hypothesis 1: The severity of ASD traits in girls with FXS will be significantly lower from that observed in boys with FXS.

Using Gender as the independent variable, SRS-2 and RBQ-2 total scores were examined using a Mann-Whitney U test. The analysis revealed there was no significant difference between male and female participants with FXS on total scores of SRS-2 symptoms (U = 172.00, z = -.09, p = .92) and RBQ-2 (U = 136.50, z = 1.03, p = .80). Subsequently, a series of Mann-Whitney U tests were conducted using gender as the independent variable and subscales from the SRS-2 and RBQ-2. However, no statistically significant differences occurred between gender and any of the subscales for RBQ-2 or SRS-2 (p > .05).

Hypothesis 2: The behavioural phenotype of participants with FXS-O will not differ significantly from the behavioural phenotype of those with comorbid diagnosis of FXS+ASD.

A Kruskal-Wallis H test was conducted which showed that there was no statistically significant difference in total SRS-2 scores between participants with FXS-O and FXS+ASD $\chi^2(2)$ = 2.469, p = .291. A second Kruskal-Wallis H test also showed there was no statistically significant difference in total RBQ-2 scores between FXS-O and FXS+ASD $\chi 2(2)$ = 3.394, p = .183.

Hypothesis 3: Both social communication difficulties and restrictive and repetitive behaviours will be positively associated with intellectual disability irrespective of gender.

Using partial correlation analysis controlling for gender indicated that there were no significant associations between measure of ID (as guided by the *Wessex)* and total symptoms of SRS-2 (r. = .03, p = .81) and RBQ-2 (r. = .11, p = .43). The following figures display these non-significant findings visually. Figure 3 visually plots total scores on RBQ-2 scores and *Wessex*

scores. Figure 4 visually plots total SRS-2 symptoms and *Wessex* scores. Figure 5 and 6 plot relationship between *Wessex* and both SRS-2 and RBQ-2 scores across different age groups.

Figure 3. Relationship between Wessex score and RBQ-2 (N = 48) (N = 48)

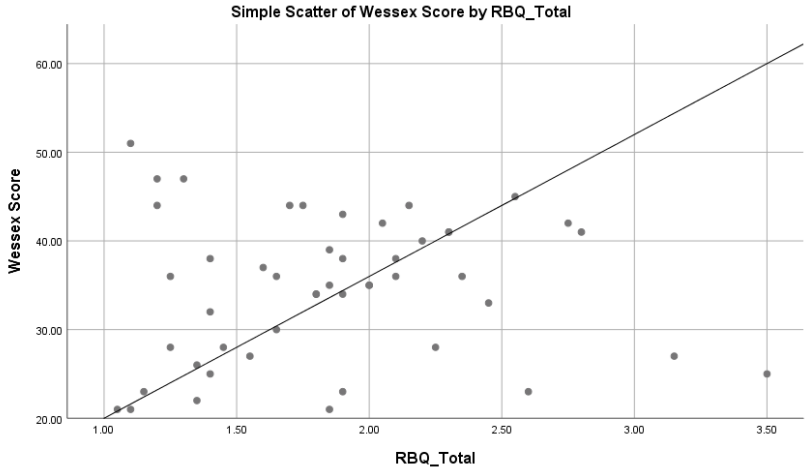


Figure 4. Relationship between Wessex score and SRS-2

Figure 5. Group scatter by Age Group on Wessex and SRS-2 scores

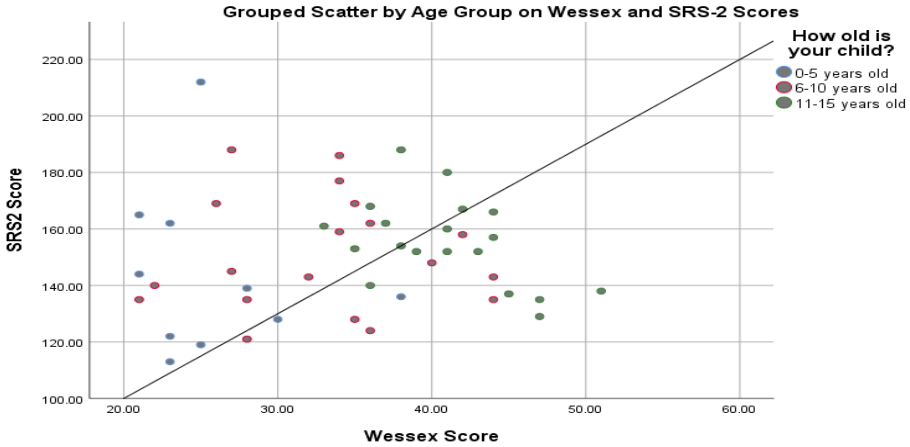


Figure 6. Group scatter by Age Group on Wessex and RBQ-2 Scores

Discussion.

This paper considered three main hypotheses. These are discussed with reference to statistically significant differences, trends and observations. The original hypotheses are considered in turn here after:

Hypothesis 1: The severity of ASD traits in girls with FXS will be significantly lower from that observed in boys with FXS;

ASD traits in girls did not differ significantly from boys and consequently the hypothesis was not accepted. Contrary to much of the literature, boys with FXS did not present significantly higher rates of ASD symptoms than girls with FXS in this study. Inspection of participants' visual profiles of the RBQ-2 and SRS-2 subscales illustrated a tendency for a higher rate of ASD symptoms in girls, although these differences did not reach significance. This supported Gallagher and Hallahan's (2012) conclusion that ASD profiles across FXS gender can present with equal severity.

Outcomes from the Wessex questionnaire suggest that this finding would be irrespective of ID. Thus, agreeing with Mazzocco's (1998) conclusion that symptoms related to ASD are not explained solely by low cognitive ability.

Girls scored higher than the males on Wessex scores, indicating less likelihood of the presence of ID, as specified by Hagerman et al., (1992). In addition, the inferential statistics and cognitive profiles do not indicate a correlation between ID, as inferred by the Wessex questionnaire, and ASD traits. This finding indicated that ASD traits were not correlated with level of cognitive ability. This is contrary to Hall et al. (2010) who found a positive correlation between IQ and the SCQ score for boys and girls.

Although Hall et al. (2010) used different measures; their finding indicates an association between IQ and symptomology, which is not found in the current study. The Wessex Questionnaire has good inter-rater reliability at subscale and item level (Richard et al., 2012) and scores between 5 and 9 have been indicated to indicate moderate ID (Bell et al, 2018). However, it does not provide an accurate indication of cognitive ability, which may explain the discrepancy with the comparison with Hall et al. (2010). These findings must be taken with caution due to the methodology.

Hypothesis 2: The behavioural phenotype of girls and boys with FXS-O *will not differ significantly from the behavioural phenotype of those with comorbid diagnosis of* FXS+ASD*.*

There was no statistically significant difference in total SRS-2 scores between participants with FXS-O and FXS+ASD, or total RBQ-2 scores between FXS-O and FXS+ASD. Statistical analysis indicated no significant difference in the total scores on these measures; the former considered to provide the most valid indication of social deficits indicative of ASD and the latter directly derived from a standardised assessment tool for ASD. Consequently, this provided evidence to accept the hypothesis. However, within the subscales making up these measures there were significant differences between FXS+ASD and FXS-O groups found in repetition; preoccupation with patterns; and rigidity and adherence to routine, which all reflect symptoms associated with repetitive and restrictive interests. The direction of significance indicated more impairment for individuals with FXS+ASD than FXS. With FXS+ASD presenting with impairment more akin to individuals with ASD, although the current study did not include an ASD group for comparison. This pattern of increased impairment from FXS (least impaired) to FXS+ASD (most impaired) supports the direction of impairment concluded by Thomas, Daffin & Hare (in submission). However, it should be noted that the low sample number in this study could affect the statistical significance of this outcome. In addition, although FXS+ASD were significantly more impaired, the FXS-O group still presented with the BP's. There could be

numerous variables that contribute to the development and severity of presentation such as: parental management; support from services; and environmental factors.

Hypothesis 3: Both social communication difficulties and restrictive and repetitive behaviours will be positively associated with intellectual disability irrespective of gender.

Repetitive and restrictive interest symptoms have been highlighted to emerge along a trajectory in FXS (Lee et al. 2016), potentially contributing to the increase in ASD diagnosis in older children with FXS and emphasising a difference in the BPs of children with FXS compared to FXS+ASD. The presence of these symptoms for participants with FXS+ASD in the current study may be accounted for by the higher number of participants within the older children age ranges with FXS+ASD. Significant differences indicated between FXS+ASD and FXS-O groups on the total score for the SRS-2 indicate the potential for use of the measure as a screening tool to identify the presence of ASD traits in FXS. However, the use of this measure in this way would require the identification of a cut off score to indicate the different presentations.

The difference between motivation measured by the SRS-2 and compared between FXS-O and FXS+ASD was also significant. The direction of significance indicated higher scores on the motivation subscale for the FXS-O group compared to the FXS+ASD group, suggesting that the latter group lack motivation. Although cause cannot be determined by the current study, it may be that FXS+ASD present with more psychiatric symptoms (Roberts et al. 2009; Rinehart et al. 2010), which contribute to the relative lack of motivation in this group.

Comparison of the total SRS-2 score across the FXS and FXS+ASD group demonstrated a significant difference in social behaviour difficulties was present, supporting the findings by Hernandez et al. (2009) for different BPs for FXS and FXS+ASD, with FXS+ASD experiencing significantly elevated difficulties. However, the lack of a significant different between groups for all items individually also provides support for the presence of ASD traits within FXS that would not meet the criteria for diagnostic cut off (Lee et al. 2016).

The descriptive statistics indicate a significant difference in diagnosis (FXS-O vs FXS+ASD) between age range groups provides support for the trajectory model of FXS+ proposed by Thomas, Daffin & Hare (in submission). As well as support for findings from longitudinal studies (Hernandez et al., 2009; Lee et al, 2016) that indicate the trajectory of FXS and FXS+ASD are different. The finding that significantly more children aged 6-10 and 11-16 had a diagnosis of FXS+ASD, than children aged 0-5, supports that symptoms of ASD in FXS develop along a trajectory with chronological age. Aged 0-5 children may not present significantly differently to FXS peers and thus not attract a diagnosis, but then go on to develop ASD traits and receive comorbid diagnosis (FXS+ASD). The lack of a significant difference between FXS and FXS+ASD in these early years could provide support for hypothesis 2, but a developmental methodology would need to be utilised to further investigate this. An increase in RRB (Lee et al. 2016) and psychiatric symptoms such as social anxiety (Roberts et al. 2009; Reiss et al. 1988; Hessl et al., 2008) in conjunction with chronological age may explain the additional percentage of FXS+ASD diagnoses in older children in the current study.

Limitations

The findings are impacted upon by the small sample size and unequal distribution of age and sex. This is particularly so for testing *hypothesis 1* which aimed to compare boys with girls. The female sample size was far below that of boys (n=9 against n=39 respectively). Although this maybe representative of the population of FXS and the noted difficulty of attracting FXS females to participate in research projects, the findings should be taken in light of this. As mentioned, it is intended for this data to be pooled with future research data which will hopefully improve the sample size and subsequent effect size. Although every effort was made to reduce the burden on the families of children with FXS, the study measures were time consuming taking on average 30 minutes to complete. This could explain the relatively high rate of partial responders, although this may also be indicative of content or design of the platform itself. The online platform enabled access to an international audience, but recruitment of participants to the level that would provide acceptable power (Cohen, 1992) and effect size was not achieved. Sixty participants, with equal allocation to groups for comparison, would have provided acceptable power. Consequently, recommendations for future research would be to re-run the statistical analysis with data from additional participants to bring the numbers up to appropriate power. Many studies in FXS hold a male dominant focus, and although this study included both sexes in the study, the number of male participants was far greater than that of female participants (N=39; N=9, respectively). This may be representative of the reduced prevalence and symptom severity in the female population.

The current study recruited participants within three different age ranges and statistical analysis considered age as a variable. The limitations of a small sample size impacted on the scope of the current study to interpret findings across a developmental trajectory. Future research utilising a

longitudinal methodology to review BPs would provide more insight into the developmental trajectory of FXS+ASD BPs.

Limitations associated with parental report in this study include the retrospective nature of their report, and the lack of formal training in identification of symptoms that the study aimed to assess, affecting reliability. Although the use of parental self-report measures is standard practice when assessing symptomology in children, best practice would be to substantiate these findings with direct behavioural observation by trained assessors. Alternative methodologies could include video analysis of BP by analysis of home video recordings, as used by Baranek et al. (2005). In addition, the self-selecting sample may provide a bias, with the potential for parents to have self-selected to participate as a result of factors such as: their contact with the FX Society meaning they were more informed, or felt more supported; or contrarily their contact with the FX Society indicated a perceived higher level of difficulty perhaps reflecting more severe impairment in their child.

The findings of the study are limited by a lack of consideration of contextual information regarding the participants, including no information about additional comorbid developmental or psychiatric diagnoses, such as Attention Deficit Hyperactivity Disorder, anxiety or depression. This is may be relevant considering the higher prevalence of comorbid diagnosis for psychiatric disorders for girls with FXS and the BP of associated symptoms (Roberts et al. 2009; Rinehart et al. 2010).

The study asked for verbal confirmation of FXS and ASD diagnosis (if present) but did not require formal clarification of diagnosis. This may have impacted on the reliability and validity of findings. However, as participants were registered with the FX Society and there were no material gains from participating, it was considered that they would be honest in their self-report. The measures used in the study were assessing for ASD traits and formal diagnosis was not required. Future research should

consider sourcing this information or confirming diagnosis as part of the research study. The generalisability of findings is limited due to the age of the participants, with future research recommended to include young people up to 25 in line with the age of intake for education provision.

Clinical and Research implications

Ultimately, this study highlights similarities between gender for FXS+ASD presentation, with ASD traits not considered solely related to cognitive ability. Clinical implications from the current study include highlighting that ID and ASD traits are not always positively correlated. Within this participant group ASD traits were as prevalent in females as in males, irrespective of ID. There was also support for the trajectory model of the development of ASD symptoms in FXS over time. Clinical implications include the need to assess for ASD later in developmental trajectory if early assessment is not indicative of ASD. Future research should expand on gender comparisons, with an additional focus on the impact of psychiatric symptoms associated with BP. A longitudinal focus will provide more information about the trajectory of symptoms and inform clinical provision for this client group.

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Paper 3.

Critical and reflective evaluation.

The focus of this section is to provide a considered critique and reflective evaluation on the work undertaken in the systematic review (paper 1) and the empirical research study (paper 2). Literature on the process of reflection highlights an anticipatory phase, where prior experiences inform future actions (Mann, Gordon & MacLeod, 2009). Discussion will consider the process undertaking this work, with reference to Mann et al (2009) anticipatory phase of reflection; then consider the strengths and weaknesses of these papers in relation to the methodology and their future contribution towards research, clinical practice, and most importantly the FXS population, their families and carers. Reflections on personal and professional development will be integrated throughout the sections of the paper.

Personal context

I travelled a somewhat unorthodox route to clinical training. My initial psychology degree was completed approximately 16 years before I commenced the journey into DClinPsy. My professional background prior to clinical training was working in Specialist Child and Adolescent Mental Health Services as a DBT and CBT therapist. Within this role I developed a surf therapy programme for adolescents diagnosed with Autism Spectrum Disorder (ASD), now in its eighth year of running. It was a difficult decision to leave this role as I thoroughly enjoyed the clinical work, meeting some truly inspiring young people on the way. The experience that I gained clinically has been fundamental to my progress through the DClinPsy, the challenge was always going to be returning to the world of research and academia. Balancing family life and the demands of the DClinPsy has been difficult at times, but always rewarding. I have three wonderful children who will be very pleased to see me emerge from my office (an old wooden tool shed in the garden) upon completion of this thesis.

As a reflective practitioner, I am familiar with iterative reflection (Schon, 1983) where my personal experience triggers exploration and learning. The proposed research project gained my interest due to the ASD traits often reported in FXS. I had experience of working therapeutically with a young woman with FXS and a number of individuals with an ASD. These experiences heightened my interest in ASD and FXS+ASD, with the passion I still hold from working in Specialist CAMHS for 10 years undoubtedly influencing the developmental focus of the research.

Review of Paper 1: Systematic review

Rationale for topic

Genetic testing has enabled accurate diagnosis of FXS, however, FXS has low homogenous behavioural properties, often perplexed by a range of ASD traits/symptoms and/or cognitive impairment. In such cases it remains unclear if this is a result of co-morbid ASD or if there is a specific behavioural phenotype for individuals diagnosed with FXS. Individuals with idiopathic Autism Spectrum Disorder (iASD) and FXS+ASD will have greater access to specialist services implemented to meet the rising need of ASD. This is often to the exclusion of some FXS individuals who do not meet the criteria for comorbid ASD diagnosis based on the outcomes of assessment tools designed for the ASD population. This has clinical implications for families as exclusion from services where specialist intervention is available for psychological and behavioural symptoms, often relies on the comorbid presence of ASD.

The project was undertaken in collaboration with the FXS society and is the second in a series with a focus on FXS. Following the general selection of FXS as the research area, and background reading to establish the current research interest and direction, phenotypic behaviour and its potential to contribute to the understanding of FXS for families and clinical practice was selected. I considered an area of extant research to systematically review which would complement and justify the direction of the empirical focus. The intended topic validated as a beneficial focus for their members by the FXS society's research board. This process was certainly aided by a carer from the FXS society who helped me develop the information sheets for the families involved.

Search strategy

The systematic review was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) and checklist (Appendix I) to ensure a methodical and transparent process (Moher, Liberati, Tetzlaff, & Altman, 2009). The initial search strategy was Ovid MEDLINE (1946-) followed by PsychINFO (1806-) and Embase (1947-). These databases were initially searched for relevant articles in August 2018. Although the discovery of the FMRI gene was in 1991 (Verkerk et al., 1991), no cut-off date was enforced at this juncture due to suggestions in the literature in the 1960's that mutations in genes on the X chromosome may be significant and termed *marker X* (Gallagher and Hallahan, 2012). A later applied exclusion criteria of articles pre-2000, highlights the insignificance of this initial consideration.

Additional papers were discovered through references of other articles. This would suggest that although I conducted a thorough and systematic literature search, my search terms were not specific enough to capture all relevant articles in the area of interest in this first stage. However, a thorough review of the papers retrieved promoted further specificity reassuring that through the overall process, all relative articles were found for synthesis.

Inclusion and exclusion criteria

An early challenge of this paper was the fairly limited knowledge of the complexities of FXS. The process of conducting a literature search was akin to *not knowing what you don't know, until you know*. As papers were being selected and read, more knowledge was gained into the area of FXS and ASD; with levels of reflection occurring within this process in order to provide a transformative experience in learning (Moon, 1999). My interest and direction often widened to the risk of being too broad with what inclusions to make. This appears to be a specific hazard when researching areas such as FXS, ASD and phenotypic behaviour (Pellicano, Dinsmore & Charman, 2014). It was also something that I could 'notice' and refocus from. After engaging in initial superficial reflective processes (e.g. noticing), I applied myself to deeper reflection during the learning process (Moon, 1999).

The inclusion and exclusion criteria required adaptation at a number of stages. The range of current research into FXS has developed considerably since the discovery of the FMRI gene and continues to do so in line with medical advances in areas such as neuroimaging. Research into BPs also delivers a wide range of sub-categories such as; neurobiological causal factors that contribute to the development of BP; the impact of family and environmental factors on BP; health needs; efficacy of psychological intervention on BP; efficacy pharmaceutical intervention on BP; diagnostic severity – carrier or full-mutation focus (repeat severity); as well as demographical focus on specifics such as children, adults and gender differences. A potential limitation is that due to the enforced specificity, not all findings from the systematic review will be applicable across the entire FXS population.

Inclusion criteria that limited the review to peer-reviewed journals were set with the aim of increase the quality of publications (Ware. 2008). As such, it could be argued that papers are more likely to get published if they report positive findings. This inclusion criteria could potentially bias positive findings

over papers that do not report non-significant findings. In contrast, support is offered that most unpublished papers tend to be from a result of lower methodological quality than published paper (Egger, et al., 2003).

Quality Assessment

An appropriate quality assessment tool should be used to ascertain the overall quality of the research paper (Sanderson, Tatt & Higgins, 2007). The methodological quality of design and analysis of the included research should be assessed to ascertain the level of bias and error (Dissemination, C.R.D, 2009). Assessing the methodological quality of research into BPs required the use of a review tool (Appendix C) developed by Cross and Hare (2013). This tool was developed in the absence of a standardised measure to assess the quality of research into BPs and was designed based on principles of Best Practice described in literature on BP methodology (Flint 1996). In the absence of a validated measure, this was deemed the most suitable option due to its previous acceptance by peer-reviewed journals in the area of FXS, ID and ASD. The original number of research papers assessed for methodological quality was N=23 (see Appendix D). The final number N = 13 were presented in Table 1 of paper 1. These research papers were also assessed against additional PRISMA methodological guidance for the domains that were appropriate for research into BPs (Moher et al. 2009). All studies included were deemed to be of good methodological quality assessed by two independent assessors.

Data synthesis

A narrative synthesis was conducted to describe the main findings from the included studies as the heterogeneous nature of: the studies; their participants, the BP being measured; their methodology; and their assessment measures meant that a meta-analysis couldn't be achieved (Dissemination, C.R.D, 2009). Furthermore, the diverse nature of the studies meant that although odds ratio (OR) or effect size (ES) was reported in some studies, it was only appropriate to conduct a review of the studies and their data analysis, rather than conduct a meta-analysis of their OR's. To control for potential bias that can occur in narrative synthesis (Valentine et al., 2017) I utilised my supervisors for synthesis of chosen studies whilst adhering to guidance on how to conduct narrative synthesis (Popay et al., 2006).

Ultimately, this systematic review was limited to cross sectional and cohort studies, with only 2 longitudinal studies. This is not so much a criticism of the research papers, rather the challenges faced in researching BPs. The review did not include intervention studies and consequently, no randomised controlled trials (RCTs) were included.

The review was limited to BPs underlying FXS and ASD presentations; however, there is also significant comorbidity with FXS and ADHD, which warrants exploring and the overlap with some BPs of ASD may provide further insight into understanding FXS. As such, the review exclusion criteria, partly constrained by the word count of the review paper, limited a much broader focus of potential underlying mechanisms involved in the development or cause of BP. As highlighted in the review, several BPs are present across FXS and iASD all of which worthy of review and inclusion. Although every effort was made to synthesise a clear and specific focus, the quantity of BPs discussed within a word constrained review paper limited the depth of discussion for each individual BP.

A further limitation in the review studies is the weighting of male participants in studies. Although it is largely accepted that males diagnosed with FXS are more impaired than females diagnosed with FXS, the increased prevalence of ID makes research into BPs more complicated. This aside, the imbalanced focus on males, as noted in the review paper, naturally creates difficulties to generalise findings across the FXS population.

Finally, to the potential limitation of reliance on parental report for assessment of ASD traits or BPs associated with the syndrome (Dillenburger et al., 2010). Some of the studies included parental report measures which is unavoidable in research into disorders or syndromes where self-report or professional assessment is not possible. To reduce the impact that this may have on the findings the methodology tool checked for validation of all measures used in the reported studies.

Future research

This review has highlighted some testable hypotheses for future research. Of note the trajectory of ASD traits in FXS appears to increase in severity with age, such as, repetitive and restrictive behaviour over time in FXS+ compared to FXS; increased severity of impairment in reciprocal social interaction; and specific pragmatic language deficits. Although this is starting to be acknowledged, more longitudinal studies are needed to transfer these findings to theory. Future research needs to address the limitations of the current review and associated studies while attempting to utilise female participants, with a longitudinal focus and minimise the reliance on parental reports of behaviour. A move towards longitudinal studies is needed to test out the trajectory hypothesis arrived at by this review. Research examining the outcomes of interventions for symptoms present across FXS, FXS+

and iASD with a focus on outcomes for underlying biological symptoms, such as the role of cortisol may be key to providing further distinction.

Implications for Theory and practitioners

There is an undeniable underdiagnoses in clinical practice of FXS+ (Klusek et al., 2014) and BP that mirror ASD can go undiagnosed, reinforcing the demand for clinical services to accept cases based on symptomatology, need and QoL as opposed to diagnosis. While clinical service provision continues to be organised based on a categorical system of diagnosis supported by DSM-IV & ICD-10, some individuals with FXS may not receive a service that could improve QoL. The developmental trajectory of FXS appears the most significant factor for consideration in clinical practice and future research as this review concludes the behavioural phenotype of FXS changes significantly with CA. Current service provision and capacity makes diagnosis for ASD likely to result from assessment at one time point in clinical practice, this may exclude children with FXS from relevant services given the trajectory of FXS+ symptomology. The current review highlighted the significance of impairment in reciprocal social interaction, restrictive and repetitive behaviours, social language difficulties for individuals with FXS+. Specifically, indicating the biological underpinning of social avoidance in FXS with indications for the clinical management of the BP.

Clinical guidelines documenting an appropriate diagnostic pathway and associated interventions for FXS+ would increase the percentage of diagnoses and subsequent relevant service access for this group. Considering the high prevalence of ASD traits, ID and psychiatric symptoms in individuals with FXS, clinical guidelines targeting these presentations would benefit from providing reference to

people with FXS and associated assessment and intervention guidelines. However, the development of such interventions relies on further clarification of the evidence base within the literature on FXS.

Review of Paper 2: Empirical study

Topic

Following the transformative learning experience (Moon, 1999) of the review of the literature in paper 1, the principle aim of the research paper was to examine the differences in the behavioural phenotype (BP) of boys and girls with a diagnosis of FXS. Research into females with FXS is limited in comparison to that of males. Comparison of males and females with a diagnosis of FXS is hypothesised to aid greater understanding of the range of BPs of ASD traits present across individuals with FXS due to the potential of controlling for cognitive ability. Aiming to compare those who have a diagnosis of FXS only (FXS-O) to those participants who have a comorbid diagnosis of FXS and ASD (FXS+ASD) three hypotheses were set:

Hypothesis 1: The severity of ASD traits in girls with FXS will be significantly lower from that observed in boys with FXS

Hypothesis 2: The behavioural phenotype of girls and boys with FXS-O *will not differ significantly from the behavioural phenotype of those with comorbid diagnosis of* FXS+ASD.

Hypothesis 3: Both social communication difficulties and restrictive and repetitive behaviours will be positively associated with intellectual disability irrespective of gender.

Ethics

The proposed project was submitted for ethical approval to the Cardiff University School of Psychology Research and subsequently approved by the Fragile X Society (FXS-UK) research board.

Methodology

Recruitment

This research paper, undertaken in collaboration with Cardiff University and the FXS-UK, was the second in a series of studies of FXS in this collaboration. In the first study by Daffin, Thomas, Hardiman and Hare (in submission) participants recruited through FXS-UK were asked if (A) they consent to be contacted at a later date to take part in both follow up studies and/or new studies and (B) if they consented to their data from the original study being made available to other researchers working on FXS under the supervision of Dr Hare.

Access to data already gathered by Daffin et al., was dependent on Cardiff University ethics approval and consent provided in the original study by participants. The aim was to ensure that participants who have already dedicated their time and consented to their data being utilised were relieved from the potential burden of being contacted with a request to complete measures already stored on data base. In order to build upon the data, set by Daffin et al., this research paper mirrored some of the methodology utilised, so data could be merged into a single data set.

As it likely that this collaboration will continue following this project, participants involved were asked if they consented for their data from the current study, to be made available to other researchers working on FXS under the supervision of Dr Dougal Hare. All participants consented to this, as well as their details being stored for future contact which is very positive for future research in this collaboration.

Participants

The on-line link for the Qualtrics survey was distributed via the FXS-UK and its associated media outlets such as Facebook and Twitter and made available for twelve weeks. Additional effort was made to specifically promote the study to reach out to female participants with FXS. Additional tweets, Facebook posts, and further promotion through the FXS society were made. Completed data was returned on 39 boys with FXS and 9 girls with FXS, but total sample number was still relatively low. This was significant for the specific aim of comparing male and female participants, with full collected data only available for 9 females.

Sample size

Although a sample of N=1500 parents of children with a diagnosis of FXS were potentially be accessible via the FXS Society membership database, a relatively low number of participants actually completed the on-line platform. A percentage of the accessible parents' children would no longer be under 16. With hindsight, it would be more inclusive to raise this to 25, as per the inclusion criteria for studies in paper 1. This would fall in line with the educational provision as well as offer a greater range of data for focus on the developmental trajectory of ASD traits. Changing the age range was not possible within the time constraints as it would involve additional ethics approval. Although this reduced the power of significance for these findings, the time and effort the participants dedicated to completing the measures has contributed to some very interesting findings and will also continue to benefit future projects in this series of studies. I would expect that as more data is added from these planned studies, the effect size will become more significant. Furthermore, a total of 92 responders registered for the platform but duplicates (N=1) and non-complete measures (N=43) were removed which drastically reduced the total. Future projects in this series should consider the length of time

required to complete the platform (between 20 - 25 minutes) as well as the order of the questions contained which is discussed in the measures section.

A final consideration would be the inclusion of international participants, although not excluded from this study, a link between FXS-UK and overseas equivalent couldn't be achieved. In the final week of this study, I was contacted by a parent of a child with FXS who lived in America (formally from the UK) who would be willing to promote the following study in the series through her membership in an FXS society in America. She kindly accessed the link to our study and provided recommendations to adapt so that the language would be suitable for both UK and American parents. This feedback will be utilised for the following studies.

Measures

The measures utilised in this study included the; *Social Responsiveness Scale – second edition (SRS-2*; Constantino and Gruber, 2012); the *Repetitive Behavioural Questionnaire 2* (*RBQ-2*; Leekam et al., 2007) and the *Wessex Questionnaire* (Kushlick et al., 1973).

Validity of the measures used in empirical research is paramount. As previously mentioned, measures chosen for my empirical paper had already been decided due to benefit of maintaining consistency in the data being collected for the series of studies focussing on FXS. However, justification and appropriateness for their use in this study was still assessed. The measures were evaluated for efficacy with the target population, whilst considering the aims of the study and whether these measures would be appropriate in providing the specific data needed to test the given hypotheses. In addition, peer accepted studies utilising these measures were sought (Wolfenden, Wittkowski, Jones, Rust, & Hare, 2019; Moss, Oliver, Arron, Burbidge, & Berg, 2009). Standardisation such as target age and the

methodology involved in developing the normative data was checked, as well as the specific process required to ensure accurate scoring, interpretation and reporting of the findings (as outlined within the measures).

I purchased the *SRS-2* so that the study was licenced to use and report on the sub-categories. The *SRS-2* is also sensitive to milder autism spectrum conditions (ASC) and provides a quantitative score for comparison across settings and against norms for autistic social impairment. This sensitivity is valuable when evaluating the presence of symptoms/BP that don't meet the threshold required on a subjective yes or no evaluation. In addition, the norms for the *SRS-2* were established through different raters, providing a more robust inter-rater reliability of the established norms. The school-age sample included 2,025 ratings of 1,014 children across 16 age levels.

The *SRS-2* provides a total score, the most reliable measure for social deficits related to ASD, and subscale scores. The measure had good consistency, validity and reliability data: Strong *internal consistency* was found across gender and age and across clinical subgroups; *Content validity* was strong with the items reviewed by experts representing various fields including special education, psychology, paediatrics, child neurology and psychiatry, and parents of children on the autism spectrum; *Descriptive data* and *internal consistency* were assessed along with mean differences across diagnostic categories associated with ASD. The clinical sample obtained raw scores considerably higher (M = 106.6) than those who represented the control (M = 24.6): A large effect size (Cohen's d = 2.7) was reported. Ultimately, the SRS-2 does well in identifying those with and without characteristics of ASD.

What I like about the *SRS-2* is that it's a validated for its measurement of impairment on a quantitative scale of severity, therefore seeing ASD symptoms on a spectrum as opposed to other measures that

provide a yes or no decision about the presence of a symptom. The ability to measure the severity of social impairment is important for clinical practice as even mild degrees of impairment can have significant effects on social functioning. This fits with much of the literature presented throughout the systematic review and empirical paper that concluded with the need to focus on symptoms rather than categorisation. Due to the reasons outlined above, the SRS-2 was deemed an appropriate measure for paper 2.

The *RBQ-2* is a four-factor measure which has 20 items, developed to broadly measure unusual sensory interests, repetitive motor movements, rigidity/adherence to routine and preoccupations with restricted patterns of interests based on based on items from Diagnostic Interview for Social and Communication Disorders (DISCO: Wing et al., 2002). It was developed using two geographical subsamples and reports good internal consistency (alpha = 0.85), inter-item validity, and across samples reliability validity. The coefficient alpha statistics for each of the four sub-scales (repetitive movements alpha = 0.80; rigidity alpha = 0.75; preoccupations alpha = 0.72; and sensory interest alpha = 0.66) suggest acceptable internal consistency. Originally validated for use with 2-year-olds, the RBQ-2 has gained acceptance as an appropriate measure for use with children and adolescents with ASD with good internal consistency across the whole scale (a=.86) and for both RSMB (a=.79) and IS (a=.83; Lidstone et al. 2014).

The *Wessex Questionnaire* (Kushlick et al., 1973) a questionnaire used to assess several dimensions of ability in children and adults with ID. Comprised of five subscales with a total of 15 items *continence* (4 items)*, self-help skills* (3 items)*, mobility* (2 items)*, speech* (1 items), *literacy* (3 items), *vision and hearing* (2 items) items are scored on a scale from 1 to 3, higher scores indicating a greater level of ability. It has good efficacy for use in large scale questionnaire studies with good inter-rater reliability at both sub-scale and item level for adults and children (Richards et al 2012).

Demographical questions, as well as questions relating to health and support needs based on work by Bromley, Hare, Davison and Emerson (2001) were additional items added to the measures in order to collate a depth of data for the series of studies in this collaboration. The data collected on the health need of these participants was not focal to the aims this research paper but was an essential component of the Daffin et al (In preparation). It is possible that due to the order of the questions on the platform, health need questions coming first, that data collection on questions for the RBQ-2, SRS-2 and Wessex may have been hindered. Partial completers who didn't complete the entire questionnaire to the end, due to the order often missed completing questions on the ASD measures.

Data analysis

The findings are impacted upon by the small sample size and unequal distribution of age and sex resulting in non-parametric analysis. Although every effort was made to reduce the burden on the families of children with FXS, the study measures were time consuming taking on average 20-25 minutes to complete. This could explain the relatively high rate of partial responders (N=42 incomplete data, participants removed), although this may also be indicative of content or design of the platform itself. The online platform enabled access to an international audience, but recruitment of participants to the level that would provide acceptable power (Cohen, 1992) and effect size was not achieved.

Implications for research and clinical practice

Within this participant group ASD traits were as prevalent in females as they were in males, irrespective of ID. Limitations aside, analysis of the data produced some significant results promoting the need to increase the research interest in females with FXS (with or without ASD diagnosis). There was also support for the trajectory model of the development of ASD symptoms in FXS over time. Clinical implications include the need to assess for ASD later in developmental trajectory if assessment earlier on has not indicated ASD. Future research should expand on gender comparisons, with an additional focus on the impact of psychiatric symptoms associated with BP. A longitudinal focus will provide more information about the trajectory of symptoms and inform clinical provision for this client group.

These findings can form the basis of direction for the following studies in this collaboration. Data can be stored and utilised as consent has been provided. Participants have also provided consent to be contacted again which provides an opportunity to conduct a longitudinal evaluation of these participants over more than one time point.

Personal and professional reflections

Through my experience of 1:1 working with a brilliant and inspiring young woman who had a diagnosis of FXS, my professional interest in FXS had been activated. This was heightened further when attending a conference for FXS research in Edinburgh. With international experts in the research field of FXS presenting, the conference emphasised the global interest in FXS, as well as the significance and range of focus. The highlight of the conference was a talk by a parent of two boys diagnosed with FXS. He provided an open and honest account of the difficulty's children with FXS and their families face. He balanced this by presenting the individual strengths and characters of the boys, whilst highlighting the impact that research has had and continues to have within the area of FXS. Whether it has been individuals with FXS, families of these individuals, or professionals with a special interest in FXS, the passion and drive of all those I have encountered, triggered personal reflections that shaped my professional focus throughout this research project.

Although this empirical paper didn't require face-to-face contact with the participants, reading some of the comments in the completed measures was emotive. Especially when related to difficulties in accessing specialist services for these children and families, often exclusion based on subjective and arbitrary categorisation criterion. I can only imagine how difficult it would be if one of my children was unable to access specialist services that could improve their quality of life. I didn't foresee the frustration of feeling powerless to intervene and found myself reflecting on the different contributions a clinical psychologist can make. Conducting research to contribute to the development of theory to guide clinical practice was a change to the direct 1:1 clinical intervention that I am experienced in. Although the findings from these papers have clear methodological limitations, they will contribute to the literature. They highlight the need to have equitable focus on females diagnosed with FXS and add justification for future research to take a developmental trajectory with regards ASD traits in FXS. Ironically, I'm not sure if answering the question of whether ASD traits in FXS represents true ASD

would be beneficial for the FXS population, as it could contribute to exclusion from current service provision for ASD.

Research dissemination

Upon request the research will be made available to any of the participants who have been involved in the project. It will also be shared will with the UK Fragile X Society for the benefit of all their members. Outcomes will also be presented at their annual UK Fragile X Conference. The research will also be disseminated at the UK Annual Seattle Club Conference and at the European Conference on Intellectual and Developmental Disabilities conference. The results will also be disseminated within the School of Psychology at Cardiff University and the Department of Clinical Psychology. It has been agreed to present the findings at the NHS Wales Learning Disability Directorate Special Interest Group (SIG) in June of this year. The findings will be disseminated in a user friendly and accessible way via the Fragile X Society and its social media outlets. This will involve the creation of a short video and a one-page leaflet presenting the results and implications. For service level engagement an SBAR format will also be created. It will also invite members of the public to comment on the findings. These will then inform future research and further dissemination.

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Appendix A

JADD: instructions for authors

Instructions for Authors

EDITORIAL PROCEDURE

Double-Blind Peer Review

MANUSCRIPT FORMAT

All JADD manuscripts should be submitted to Editorial Manager in 12-point Times New Roman with standard 1-inch borders around the margins.

APA Style

Text must be double-spaced; APA Publication Manual standards must be followed.

As of January 20, 2011, the Journal has moved to a double-blind review process. Therefore, when submitting a new manuscript, DO NOT include any of your personal information (e.g., name, affiliation) anywhere within the manuscript. When you are ready to submit a manuscript to JADD, please be sure to upload these 3 separate files to the Editorial Manager site to ensure timely processing and review of your paper:

A title page with the running head, manuscript title, and complete author information. Followed by (page break) the Abstract page with keywords and the corresponding author e-mail information.

The blinded manuscript containing no author information (no name, no affiliation, and so forth).

The Author Note

TYPES OF PAPERS

Articles, Commentaries Brief Reports, Letters to the Editor

The preferred article length is 20-23 double-spaced manuscript pages long (not including title page, abstract, tables, figures, addendums, etc.) Manuscripts of 40 double-spaced pages (references, tables and figures counted as pages) have been published. The reviewers or the editor for your review will advise you if a longer submission must be shortened.

Special Issue Article: The Guest Editor may dictate the article length; maximum pages allowed will be based on the issue's page allotment.

- Commentary: Approximately 20-25 double-spaced pages maximum, with fewer references and tables/figures than a full-length article.
- A Brief Report: About 8 double-spaced pages with shorter references and fewer tables/figures. May not meet the demands of scientific rigor required of a JADD article – can be preliminary findings.
- A Letter to the Editor is 6 or less double spaced pages with shorter references, tables and figures.

  Style sheet for Letter to the Editor:
- A title page with the running head, manuscript title, and complete author information including corresponding author e-mail information
- The blinded manuscript containing no author information (no name, no affiliation, and so forth):-

  - 6 or less double spaced pages with shorter references, tables and figures

  - Line 1: "Letter to the Editor"

  - Line 3: begin title (note: for "Case Reports start with "Case Report: Title")

  - Line 6: Text begins; references and tables, figure caption sheet, and figures may follow (page break between each and see format rules)

REVIEW YOUR MANUSCRIPT FOR THESE ELEMENTS

1. Order of manuscript pages

Title Page with all Author Contact Information & Abstract with keywords and the corresponding author e-mail information.

Blinded Manuscript without contact information and blinded Abstract, and References

Appendix

Figure Caption Sheet

Figures

Tables

Author Note

MANUSCRIPT SUBMISSION

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

TITLE PAGE

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

ABSTRACT

Please provide an abstract of 120 words or less. The abstract should not contain any undefined abbreviations or unspecified references.

KEYWORDS

Please provide 4 to 6 keywords which can be used for indexing purposes.

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

BODY

- The body of the manuscript should begin on a separate page. The manuscript page header (if used) and page number should appear in the upper right corner. Type the title of the paper centered at the top of the page, add a hard return, and then begin the text using the format noted above. The body should contain:
- Introduction (The introduction has no label.)
- Methods (Center the heading. Use un-centered subheadings such as: Participants, Materials, Procedure.)
- Results (Center the heading.)
- Discussion (Center the heading.)

HEADINGS

Please use no more than three levels of displayed headings.

Level 1: Centered

Level 2: Centered Italicized

Level 3: Flush left, Italicized

FOOTNOTES

Center the label "Footnotes" at the top of a separate page. Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

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The first paragraph contains a separate phrase for each author's name and the affiliations of the authors at the time of the study (include region and country).

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REFERENCES

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

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  Harris, M., Karper, E., Stacks, G., Hoffman, D., DeNiro, R., Cruz, P., et al. (2001). Writing labs and the Hollywood connection. Journal of Film Writing, 44(3), 213–245.

- Article by DOI

  Slifka, M. K., & Whitton, J. L. (2000) Clinical implications of dysregulated cytokine production. Journal of Molecular Medicine, https://doi.org/10.1007/s001090000086

- Book

  Calfee, R. C., & Valencia, R. R. (1991). APA guide to preparing manuscripts for journal publication. Washington, DC: American Psychological Association.

- Book chapter

  O'Neil, J. M., & Egan, J. (1992). Men's and women's gender role journeys: Metaphor for healing, transition, and transformation. In B. R. Wainrib (Ed.), Gender issues across the life cycle (pp. 107–123). New York: Springer.

- Online document

  Abou-Allaban, Y., Dell, M. L., Greenberg, W., Lomax, J., Peteet, J., Torres, M., & Cowell, V. (2006). Religious/spiritual commitments and psychiatric practice. Resource document. American Psychiatric Association. http://www.psych.org/edu/other_res/lib_archives/archives/200604.pdf. Accessed 25 June 2007.

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Appendix B

Methodology assessment tool

Methodology quality assessment tool. Cross & Hare (2013).

Research paper assessed for:	Score
1. Control group	0 = no control group, 1 = comparisons between non-genetically distinct groups or utilise standardised assessment tools, 2 = genetically distinct control group.
2. Sample size	0 = fewer than 15 participants, 1=15+, 2=30+.
3. Recruitment	0 = participants selected by clinician(s), 1 = participants recruited either through charity or medical clinic, and 2 = multiple methods, multiple clinics or multiple charities are used for recruitment.
4. Syndrome diagnosis	0 = syndrome diagnosis based on self-report, 1 = diagnosis based on physical features or sibling diagnosis, 2 = diagnosis based on appropriate genetic/enzyme testing.
5. Methodology	0 = no validated measures are used, 1 = use validated and/or standardised assessment tools, 2 = validated and/or standardised measures are used alongside new measures, observations or other methodology.
6. Considerations for development	0 = participants are compared 'en mass', 1 = the study considers age as a variable for at least one aspect of development or behaviour, 2 = age is considered as a variable in relation to development and behaviour (or all areas investigated).
7. Appropriate statistics/ comparisons.	0 = data not analysed, 1 = descriptive statistics are used, 2 = appropriate comparative/correlative statistics are reported.

APPENDIX C

Initial methodology screening table N=23

Author/ Year/ Country	Score	Study Aims	Control Group	Sample Size (age range)	Recruitment	Diagnosis	Methodology	Developmental Factors	Stats	Findings
1.Crawford, Moss, Anderson, Oliver, & McCleery, 2015		Examine individuals with FXS and ASD if they spontaneously discriminate between facial expressions eg time looking at eyes mouth	FXS = 13 ASD – 15 TD – children = 16	1 f (mean 19.7 st dev 9) 3 f (11 – std 3.4.8) 18 f (7.13 – std 1.61) 12 adults (21.92 std2.97)	Cerebra centre for fxs and asd Community outreach recruitment campaign TD children infant & child database TD adults research pool university	ASD ADOS FXS database genetic	SCQ (rutter 2003) Vineland ABS 2nd edition Survey interview form (sparrow et al 2005) Demographic Q'aire Mac Brain face stimulus set (Tottenham et al 2009) eye tracking task	Vineland instead of IQ due to age range and ability Difference in chronological age considered in ANCOVA statistical analysis between happy and disgust preference	Shapiro-wilk test for normality. Paired samples t-tests; (time looking at faces on the left and faces on the right); time spent looking at happy vs neutral and disgust vs neutral). Independent samples t-test compared asd vs fxs happy and disgust preference. ANCOVA with CA as co-variant.	FXS & ASD participants higher proposition of looking disgust over neutral but not happy over neutral faces Ancova found no difference between happy and disgust preference according to CA
	14/14		2	2	2	2	2	2	2	
2.Farzin et al., 2006		Compare prevalence of ASD and ADHD symptoms in boys with pre-mutation presenting as probands, in brothers with the pre-mutation who do not present as probands and normal brothers of premutation and / or full mutation carriers	43 male children (14 probands; 13 nonprobands; 16 male siblings of individuals with FXS who were negative for mutation)	Mean age = 10.3 +/- 5.0 14 probands (Age 9.3 +/- 4.8) 13 nonprobands (Age 11.5 +/- 5.1)	24/43 University of California, Davis or 19/43 La Trobe University Australia	Molecular testing Documentation of pre-mutation allele using polymmerase chain reaction (PCR) and southern blot analysis Control subjects confirmed to be negative for premutation Nonprobands identified through	Molecular testing Cognitive & behavioural assessments (Weschler preschool & primary scale of intelligence 3rd edition; Connors Parent Rating Scale revised CPRS-R:S; ADHD symptoms confirmed by DSM-IV-TR diagnostic criteria by authors; structured	Effects of age and IQ examined by comparisons using the Kruskal-Wallis test, which revealed no significant difficulties (but 4 probands and 4 non probands had below average FSIQ <85)	Descriptive stats Results compared Kruskal-Wallis compared SCQ and Connors standardised T-score dependant variables and sample group as independent variable = significant overall group differences on SCQ and Connors T scores. Follow up comparisons of each pair of groups was done using Mann-Whitney =	Study indicates pre-mutation carriers (even if don't present clinically) may be at increased risk of asd and/or symptoms of ADHD. If premutation is identified further assessment of symptoms of ADHD social deficits or learning disabilities

						pedigree analysis and cascade testing in FXS families after proband was found	medical interview; For ASD: Lifetime form of the Social Communication Questionnaire SCQ; Autism Diagnostic Observation Scales ADOS or ADI used to assess according to DSM-IV-TR Parent interview & school records		non proband boys with pre-mutation had higher SCQ than controls; no sig diff in Connors scores; difference nonproband vs proband with pre-mutation showed probands had higher SCQ scores and difference on Connors approached significance; control and proband groups showed highly sig diffs on SDQ and Connors. X2 analysis showed significant diff between proband who took medication (87%) and controls (0%) but no diff non-probands (17%) and controls. Pairwise spearman's rho – no sig correlation between	
	14/14		2	2	2	2	2	2	2	
3.Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010		To examine; the presence of autistic behaviours; the profile of autistic behaviours in FXS by comparing rates	No control group but utilised standardised assessment tools (e.g. compared to mean item scores for	120 children (aged 5-25)	Across US through nation FX foundation; flyers; local contacts; research website	Using PCR and southern blot DNA analysis.	ADOS & SCQ & IQ Also used 'other' methodology: FX FMRP protein.	Age considered as a variable for; autistic behaviour; IQ; and all areas investigated	2 sample T-tests Multiple regression model (RM)	Boys & girls show lower rates of impairment on communication and interaction items than the reference autism samples.

		of individual symptoms shown by individuals with FXS to the reference autism samples provided on measures; and to determine whether IQ and autistic behaviours were associated in FXS while controlling for age, medication and FMRP levels.	autism sample contained in the ADOS manual.					However, no developmental trajectory, data collected at one time point.		IQ was significantly negatively associated with the SCQ total score in boys and girls with FXS controlling for age, medication and FMRP levels
	11/14		1	2	2	2	2	0	2	
4.Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009		Investigate eye gaze avoidance is associated with high levels of hyperarousal during social interaction with 50 boys and girls with FXS aged 5-20 years during 25 minutes intensive social interaction with an unfamiliar experimenter	50 pairs of sex matched siblings (26 male / 24 female with FXS & 50 Same sex TD biological siblings unaffected)	26 M / 24 F pairs Sex matched sibling closest in age Age 5-20 30 children (60%) older same sex siblings 19 children (38%) had	Across USA and Canada National FXS foundation; flyer to SIG, local contacts & research website	Genetic testing CGG repeat lengths <40; none of the siblings were carriers of pre-mutation of full mutation	Heart rate monitoring device whilst watching 10 min video (last 5 mins used in analysis); 25 minutes conversation with researcher whilst being requested to maintain eye contact ABABA design to variate proximity of the researcher to the participant 0-2m; observations recorded using software to code	Sex matched with closest sibling in age; chronological age was included as a covariate in 2dfWald test	FXS vs TYP compared using mixed methods regression model with random effects using XTREG in STATA 10 (Stat Corp College Station TX) 2-dfWald test to inspect both interactions simultaneously; chronological age and activity counts at time T were included as covariates	FXS significantly higher heart rates, lower vagal tone & lower heart rate variability estimates at baseline and during social interaction vs TYP Eye gaze avoidance sig higher level in FXS; but behaviour decreased slightly over the session and did not seem to be associated with cardiovascular activity. Girls with FXS higher levels of FXMRP were associated with higher (and more typical) heart rate variability.

				younger same sex sibling. 1 pair non-identical twin			multiple behaviours		Separate analysis for male and female subjects	Conclusion both sympathetic and parasympathetic nervous Systems dysregulated in FXS.
	14/14		2	2	2	2	2 ?validated	2	2	
5.Hatton et al., 2006	8 but with queries ??? quality of study write up?	ASD behaviour in children with FXS to determine prevalence of Autistic behaviour in FXS; examine the stability of ASD ratings over time (repeated measures); assess association between FMRP and autistic behaviours (association between protein and autistic behaviour)	179 children with FXS Subset: 116 children with 396 repeated observations Subset 83 children to examine impact of FMRP comparison between non genetically distinct groups	179 children Age in months: Females 49.4 (SD 29.3) Males 51.9 (SD 27.9) Total 51.6 (SD 27.4)	Data from larger longitudinal study. Recruitment strategy not described.	FMRP collected on one occasion for 83 children – however this isn't specified that DNA testing has taken place to confirm the diagnosis	Childhood Autism Rating Scale (CARS) – assessments completed twice yearly or annually in schools or children's homes	Age effects detected for CARS scores and changes over time	Descriptive stats using cross sectional data from first CARS Longitudinal data analysed using HLM (hierarchical linear modelling is an ordinary least square regression based analysis that takes hierarchical structure of the data into account) A baseline model was fit predicting CARS scores from age	29% of the sample of 129 scored at or above the cut off for Autism; CARS scores increased slowly but significantly over time; lower levels of FMRP were associated with higher levels of autistic behaviour
	/14		?1	2	-	?	1	2	2	
6. Hernandez et al., 2009			This one FXS - ASD FXS no ASD							
7.Hessl et al., 2001		In home evaluation of 120 children (80 M/40 F) with FXS full mutation and unaffected sibling;	Unaffected siblings (62 girls / 58 boys)	120 children (80 M/40 F; 5 girls & 9 boys were mosaic)	Recruited from FXS register; advertisements; FXS newsletter; National FXS email; Stanford research website;	DNA testing	Southern Blot analysis performed by Kimbell Genetics FMRP analysed	Child IQ was considered to account for variation in the behaviour attributed to	Multivariate analysis of variance. Multiple regression analysis, to examine bivariate correlations between planned	Boys with FXS effectiveness of education and therapeutic services and parental psychological problems preicted internalising and

		including measurements of FMR1 protein, quality of home environment, maternal & paternal psychopatholog y, effectiveness of education, therapeutic services & child behavioural problems		6-17 years old FXS mean = 10.76 (SD = 2.83) Unaffect ed siblings = 11.2 (SD = 3.10)	referrals from researcher; national FXS foundation clinicians & family		IQ: WISC-III (ages 6-16) Behaviour: Child Behaviour Checklist Parental: The Symptom Checklist – 90 revised Home: Home observation for measure of the environment (HOME) Economic status: Parental report of gross household income annual / divided by medium household income in the area Education: Special curriculum opportunity ratifying scale (SCORS)	developmental disability	independent variable and dependent variables. Variance in behaviour problems hierarchical. multiple regression analysis separately for boys with FXS, girls with FXS and comparison siblings. Hierarchical approach to bio/genetic vs environment: Step 1: Biological characteristics (gender IQ & FMRP) Step 2: Environmental (quality of home – HOME; parental psychopathology SCL-90-R mean score; effectiveness of SCORS correlation). Regressions and follow up analogous regression were performed on withdrawn, anxious/depressed, thought & attention problem subscales of CBCL	externalising problems; quality of the home environment predicted autistic behaviour Girls with FXS results emphasises sig effect of FMRP on behaviour esp social withdrawal and anxious behaviour Findings link FMRP expression to behaviour; also emphasises significance of home and school based environmental variables in the neuro behavioural phenotype
	14/14		2	2	2	2	2	2	2	

8.Hustyi et al., 2015		Relationship between autistic symptoms and independent living skills in adolescents and young people with FXS	35 age and IQ matched controls	70 individuals with FXS plus 35 controls FXS: 35 males; 35 female aged 15-25 35 controls = 16 male; 19 female	NIH funded longitudinal study investigating functional outcomes and neuro imagining of adolescents and young adults with FXS. FXS participant recruited via advertisements; community media and national FXS foundation. Control =community media; state run agencies for individuals with developmental disabilities	Confirmed diagnosis of FXS (Southern Blot DNA analysis) Controls were screened to confirm no FXS and no diagnosis of unknown genetic disorder	Independent living skills: Independent living scales Autistic symptoms: ADOS (Lord et al. 2000)	Matched aged and IQ controls Balance groups on degree of autistic symptoms meant that 7 males with highest autistic symptomology on ADOS excluded Control for IQ conducted correlation analysis in which IQ was included as control variable	Correlation analysis of the data for autistic symptomology and independent living skills by pearson correlation between total score on ADOS and raw scores on ILS Control for IQ conducted correlation analysis in which IQ was included as control variable To compare correlation coefficients between groups Fishers r-t-z transformation was used.	Higher levels of autistic symptomology associated with lower levels of competence in independent living skills in individuals with FXS but not in control groups Data indicates relationship between autistic symptomology and independent living skills was syndrome specific
	14/14		2	2	2	2	2	2	2	
9.Kau et al., 2004		Distinctive SBP of boys with FXS, if so does age effect SBP when compared FX cohort	3 groups: FXS with ASD; DLD with ASD; & idiopathic ASD	55 – FXS 22 – DLD 11 - Idiopathic	Kennedy institute 'word of mouth' American ASD society	Genetic testing. Southern blotting techniques.	S/VM	No developmental trajectory, data collected at one time point. However, SBP stratified by age	DS BC WC	FXS & ASD & more generally SBP is a distinctive sub-phenotype amongst boys with FXS
	11/14		= 2	= 2	= 2	= 2	= 1	= 0	= 2	
10.Kaufmann et al. 2017)		Impact of ASD diagnosis in	2 groups:	Cross sectional	(FORWARD) Multisite	Full details for enrolment for	Clinician & parent report	Data analysis	X2 tests for association; t tests;	Conclusions: Greater frequency of seizures

		clinic based FXS population Compared: frequency of seizures occurring at any age; sleep problems enquiring meds or treatment; cooccurring behavioural problems; (including attention deficits, hyperactivity, hyper sensitivity, over reactivity, anxiety, obsessive compulsive disorder, perseverative behaviour, mood swings, depression, irritability, aggression)	Aged 3-11 n=348 Aged 12-21 n=199	data on 713 subjects with FXS (from sept 2012-august 2014); following exclusions almost 600 eligible	observational study that includes a registry and longitudinal database using standardised clinician and parent report data submitted by 25/27 specialty clinics across the US affiliated with Fragile X clinic and research consortium full details for enrolment for FORWARD presented in Sherman et al. (2017) → Recruitment is clinic based, with individuals attending specialty clinics approached	FORWARD presented in Sherman et al. (2017) Sherman reports that participant enrolled on longitudinal database are a subset of those on the registry who have been diagnosed with the full mutation ASD diagnosis from FORWARD clinician report	form (yearly clinic visit) Parents asked about provision of services to participant at school years (special ed; speech & language, OT, sensory integration, physical therapy, psychological/behavioural programme, social skills therapy, program, tutoring or ABA) 3 standardised parent report measures: Social responsiveness scale second edition (SRS-2); social communication questionnaire (SCQ) Aberrant Behaviour Checklist, Community edition.	conducted for child group (3-11) and adolescent / adult group (12-21), 'because the impact of the asd diagnosis on certain parameters may differ by age'.	& a multiple logistic regression to examine association between clinical and other factors with asd status Multiple regression for multivariate analyses related to use of services and ASD status	and certain behavioural cooccurring conditions eg. Aggression/disruptive behaviour in indivs with FXS and ASD have considerable impact on management; underuse of behavioural services in some indivs is concerning considering core nature of ASD and associated challenges
	13/14		1	2	2	2	2	2	2	
11. Klusek, J., Martin, G. E., & Losh, M. (2014).		Consistency between research and clinical								

		diagnoses of autism among boys and girls with fragile X syndrome.								
12.Lee, Martin, Berry-Kravis, & Losh, 2016		How manifestation of ASD related phenotypes may change over development: characterise ASD phenotypes in boys and girls with FXS over development and compare individual component phenotypes among boys with FXS vs boys with idiopathic ASD over time (Time 1 and Time 2 = 2.5 years apart)	2 groups: FXS group (31 M; 34 F) ASD-O group (19 M) Time1: FXS-girls = 34 FXS-O =24 FXS-ASD = 10 FXS-boys = 31 FXS-o=14 FXS-ASD=17 ASD-boys=19 Time 2: FXS-girls=34 FXS-o=20 FXS-ASD = 14 FXS-boys = 31 FXS-o=6 FXS-ASD=25 ASD-boys=19	65 boys and girls with FXS: FXS girls n=34; age = 8.96 (SSD=3.39) FXS boys n=31; age = 8.97 (SD = 2.51 19 boys with ASD-O Age = 9.08 (SD=2.31)	Advertisements at genetic clinics and physicians' offices, advocacy groups and participant registries	FXS: Genetic testing not explicitly stated, but recruitment from genetic clinics. ASD: Previous clinical diagnosis confirmed through direct assessment wit ADOS and/or ADI-R and no known ASD related monogenic disorders	ASD classification: ADOS and ADI-R Cognitive & language abilities: Leiter International Performance Scale Revised; Expressive Vocabulary Test; Peabody Picture Vocabulary test 3rd or 4th ed; Pragmatic judgement subtest of the Comprehensive assessment of spoken language (CASL)	Main effects of chronological age and mental age using hierarchical linear modelling (HLM) (Looked at mental age predicting symptom severity)	Compared 2 time points mean 2.5 years apart. McNemar's test of classification assessed rates of ASD and whether rates of agreement between ADOS and ADI-r changed over time. Nonparametric tests assess mean changes in classification in FXS over time; hierarchical linear modelling (HLM) and repeated measures asses changes in individual ASD symptoms in FXS over time. ANCOVAS compared ASD symptoms severity and component phenotypes in boys with FXS-O, ASD-O, FXS-ASD at both time points	Importance of adopting a developmental perspective when investigating shared behaviour features across disorders. ASD symptoms increased in FXS with age; social language impairment emerged as a potential core shared feature of FXS and ASD, which may elucidate underlying molecular genetic variation related to phenotypic variants.
	12/14		2	2	2	?	2	2	2	
13..Martin et al., 2017		Ability to signal non-comprehension across different	FXS DS ASD TD (input age)	M=121 F=81	Research participant universities, parental support	Full mutation	Cognition & Language: Brief IQ composite of the Leiter	rANCOVA = nonverbal, mental ages and	Repeated measures analysis of covariance (rANCOVA) –	Youth with FXS without ASD and those with ASD-O did not differ from TD; no sex

		types of confusing message conditions in child and adolescents with FXS, DS, ASD and TD	9 groups based on sex and diagnosis (NB. Participants with DS or TD who scored as ASD were excluded)	With and without ASD	groups, genetic clinics, childcare centres and schools.		Revised Peabody picture vocab tests. ASD: ADOS and ADI-R Noncomprehension signalling task: The Barrier Task (Abbeduto et al.)	receptive vocabulary age. Controlled for mental age	nonverbal, mental ages and receptive vocabulary age. Sex effects were assessed within diagnostic category. Follow up ANCOVAS and post hoc comparisons. Mauchley's test and Greenhouse-Geisser reported for all comparisons and conditions. Linear regression.	differences were detected in any group. Findings contribute to current knowledge of pragmatic profiles and different forms of genetically based neurodevelopmental disorder associated with ID. Youth with comorbid FXS and ASD and those with DS were less likely that TD controls to signal non-comprehension of confusing messages.
	14/14		2	2	2	2	2	2	2	
14.Martin, Bush, Klusek, Patel, & Losh, 2018		Multimethod approach to language sample analysis to characterise syndrome and sex specific profiles across FXS & ASD and FXS-O and DS and TD ASD-O	FXS & ASD n=61 (46 boys / 15 girls) FXS-O n=40 (13 boys/27 girls) DS n=42 (20 boys/22 girls) TD n=37 (19 boys / 18 girls) ASD-O n=29 (29 boys)	M=Mean 10.4 SD 2.4 F=Mean 9.3 (SD 3.8) M=9.7 (SD 3.3) F= 9.5 (SD 3.7) M=10.9 (SD 2.1) F=9.2 (SD 2.2) M=4.7 (1.0) F=5.4 (SD 2.5) M=9 (SD 2.4)	Large scale longitudinal study recruited from parent support groups, child care, schools, research registries and genetic clinics.	FXS = full mutation TD and DS groups excluded if met criteria for ASD TD = no history of developmental of language delays	ASD: ADOS Cognitive & structural language abilities: Leiter Revised Peabody picture vocab tests. Expressive vocabulary test.	Analysis controlled for non-verbal mental age, receptive and expressive vocabulary age equivalents and mean length of utterance.	Series of analysis of covariance (ANCOVA) controlling nonverbal mental age and structural language; ANCOVAs addressed group difference for M&F; cohens D (effect sizes).For each of the groups and all groups combined bi-variate correlation between hand coding and conceptually related SALT variables.	Non-continent language and perseveration were characteristics of the pragmatic profiles of boys and girls with FXS and ASD and boys with ASD only. Boys with ASD only also initiated turns les often and were more non-responsive than other groups; girls with FXS ASD ere more non responsive than male counterpart
	14/14		2	2	2	2	2	2	2	

15.McDuffie, Thurman, Hagerman, & Abbeduto, 2015)		Which current symptoms of ASD differed in boys with FXS relative to same aged boys with ASD.	FXS n=49 ASD n=39	Age: (Over all between 4 and 10 years) FXS = 7.5 (SD 2.03) ASD = 7.27 (SD 1.9)	Sample drawn from larger longitudinal study examining learning in males with FXS (n=57) and non-syndromic ASD (n=61) Recruited nationally and tested at 2 test centres.	Fxs full mutation genetic ASD – genetic screening to rule out FXS – diagnosis of asd (ADI-R and ADOS)	Nonverbal cognition: brief IQ screener; leiter international performance scale revised. ASD: ADI-R & ADOS	Chronological age matched group for all areas investigated	Mann-Whitney U test to evaluate between group differences. Descriptive stats; significance levels; corresponding effect sizes.	Boys with FXS show sig less impairment in social smiling than did age, severity and diagnostic boys with non-syndromic ASD Severity matched boys with FXS showed more impairment in complex mannerisms than boys with non-syndromic ASD
	14/14		2	2	2	2	2	2	2	
16.Oakes et al., 2016		Examine the profile intercorrelations and predictive correlated of repetitive behaviours in boys with FXS	39 boys with FXS (repeated measures design time 1/time 2)	FXS group n=39 ages 6-10 years (Mean 7.41)	Sample drawn from larger longitudinal study; recruited nationally using variety of sources including newspaper adverts, flyers, uni research.	Confirmed diagnosis of FMR1 (genetic testing)	2 time intervals (T1 & T2) At time 2: Nonverbal cognitive ability: Leiter – R Maladaptive behaviours: Anxiety and depression and Mood scale (ADAMS) ASD: ADOS At Time 2: Revised Repetitive Behaviour Scales Revised (RBS-R)	Compare time 1 and time 2;?	Freidman's analysis of variance was used to determine if repetitive behaviours differed as a function of the RBS-R subscale. Analysis followed up by Wilkoxon ranked sum test between subscale. Spearman rank correlations	Some classes of repetitive behaviours were more problematic than others eg. Ritualistic and sensory motor behaviours frequently endorsed as problematic; self injury least reported as problematic (Consistent with Wolk et al 2012)
	11/14		0	2	2	2	2	1?	2	
17.Roberts et al., 2009)		Examine environmental and nonendocrine	FXS FXS & ASD	? review table 1??	Recruited from Carolina FXS project – a series of	Full mutation based on DNA testing for all	SAS – Social Approach scales - time sensitive experimental	Groups were age matched and age was used as a	ANCOVA to examine SAS scores at initial and familiar assessment	Poor social approach and elevated baseline salivary and regulatory cortisol are discernible

		factors that convey increased risk of elevated autistic behaviour in boys with FXS. 3 related analysis 1 examination of multiple dimensions of social approach behaviours and how they vary over time; 2 invetsigation of mean levels of salivary cortisol levels; 3 examination of social approach and autistic behaviours to salivary cortisol	TD (all boys)		longitudinal studies that recruited children across US through Parent FXS list (University of N Carolina FXS registry). FXS support groups, FXS clinics and research projects	FXS participants	measure of multiple forms of social approach behaviour Adaptive Behaviour – Vineland Adaptive Behavioural Scales Cortisol - 2 samples of salivary cortisol CARS – widely used reliable measure of ASD in children	covariate when age differed	intervals (controlling for age) Regression of CARS total scores ANCOVAs to examine cortisol levels as initial and regulation assessment intervals Cortisol levels and correlation with SAS and CARS for the 2 groups with FXS were analysed using multiple regression with age controlled for.	traits that distinguish boys with FXS and ASD from boys with FXS only from TD boys Blunted cortisol change is associated with increased levels of autistic behaviours only within FXS and ASD group. Bos with FXS and ASD have distinct behavioural and neuroendocrine profiles differing from those with FXS alone and TD boys.
	12/14		2	??	2	2	2	2	2	
17.Roberts et al., 2018)		Social anxiety and FXS and association with ASD;	N=77 FXS n=59 ASD n=18	Age range 15-23 (Mean 18.18 SD 2.3)	Longitudinal multisite study	Full mutation genetically tested	-Social Avoidance scale. -Baseline cortisol level. -Anxiety & depression & mood scale (ADAMS) -ADOS -2 -Leiter international performance scale revised	Check	-Descriptive stats -Multivariate outcome analysis on covariance (ManCOVA) -Levens test of homogenity -Tukey Honestly Significant Different for multiple comparisons -Linear discriminate functional coefficient -ANOVA	Look at summary

	/14		2	2	2	2	2		2	
19.Rogers, Wehner, & Hagerman, 2001)		Examine symptoms of ASD and relationships between ASD symptoms and developmental variables in young children with FXS	3 genetically distinct groups; -27 participants ASD -23 with other developmental delays. -24 with FXS	30+ Age: 21 months – 48 months	Multiple measures.	ADI-R. ADOS. Genetic testing for FXS group but not for control group	S/VM: ADI; ADOS; Vineland; Mullen. Other measures used but not reported.	Although age closely ranged groups compared 'en mass'. No developmental trajectory, data collected at one time point.	DS BC	
	11/14		2	2	2	2	1	0	2	
20.Thurman, McDuffie, Hagerman, & Abbeduto, 2014)		Examine psychiatric symptoms in boys with fragile FXS using a parent report instrument.	41 – FXS group 41 - nonsyndromic ASD	FXS 41 males aged 4.06–10.63 years (M = 7.24, SD = 2.04). 41 participants with nonsyndromic ASD in the Full Sample ranged in age from 4.02 to 10.99 years (M = 7.46, SD = 1.76).	Large scale research project but described in detail Recruitment through 2 university sites	FXS: Genetic diagnosis for FXS ASD: screened to exclude FXS ADOS for ASD diagnosis	Leiter NVIQ SS ADOS ADAMS (2003)	?	-Friedman's Analysis of Variance (ANOVA). -Results of follow-up Wilcoxon tests -Mann Whitney U tests were used to address -Differences in psych symptoms between FXS & ASD group -Spearman correlation coefficient for within-group associations -A Fisher r-to-z transformation to determine if significant between-syndrome differences were observed in the	symptoms of manic/hyperactive behaviours and general anxiety more frequently reported for FXS than ASD. Also positive association between social avoidance and general anxiety in FXS than ASD

									strengths of association between psych symptoms	
	12/14		2	2	2	2	2	0?	2	
21.Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015a		Aims to compare the profiles of ASD relative to age (CA), nonverbal IQ, and expressive vocabulary ability between FXS and i nonsyndromic ASD	53 boys FXS 39 boys with ASD	53 boys FXS 39 boys with ASD 4-11 years in both groups	Recruitment through 2 university sites	Document of proof for Genetic testing ASD: screened to exclude FXS ADOS and ADI-R for ASD diagnosis	ADOS and ADI-R for ASD Leiter-R Non-verbal IQ Expressive Vocabulary test	Cross sectional developmental trajectories to compare ASD symptoms relative to chronological age	States uses procedures used in Thomas et al 2009. Look up	Results suggest that the onset of ASD symptoms and their developmental trajec-tories in males with FXS differ in important ways as a function of CA, nonverbal cognitive ability, and expressive vocabulary relative to males with nonsyndromic ASD.
	12/14		2	2	2	2	2	2	?	
22.Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015b		Evaluate the ability of Males with FXS, ASD & typical development to learn new words by using cue	Males with; FXS (n = 32) nonsyndromic ASD (n = 32) or TD (n = 32).	FXS: 7.29 (2.03, 4.06–10.32) ASD: 7.37 (1.87, 4.02–10.86) TD: 3.93 (1.09, 2.05–5.8)	Recruitment through 2 university sites	Document of proof for Genetic testing ASD: screened to exclude FXS ADOS and ADI-R for ASD diagnosis	Leiter-R Peabody picture vocab test 4th edition ADAMS ADOS SCQ	CA as predictor variable in all areas investigated	-Fast mapping task. -Wilcoxon signed-rank tests to compare each groups performance -Mann-Whitney U test for between group differences -Aligned Rank Procedure to compare successful search performance between ASD & FXS after controlling for ASD severity -Chi square for evaluating group level findings could be replicated -One sample Wilcoxon for	Performance for all groups exceeded chance-levels in both search conditions. In the Successful Search condition, participants with nonsyndromic ASD performed similarly to participants with FXS after controlling for severity of ASD. In the Unsuccessful Search condition, participants with FXS performed significantly worse than participants with nonsyndromic ASD, after controlling for severity of ASD.

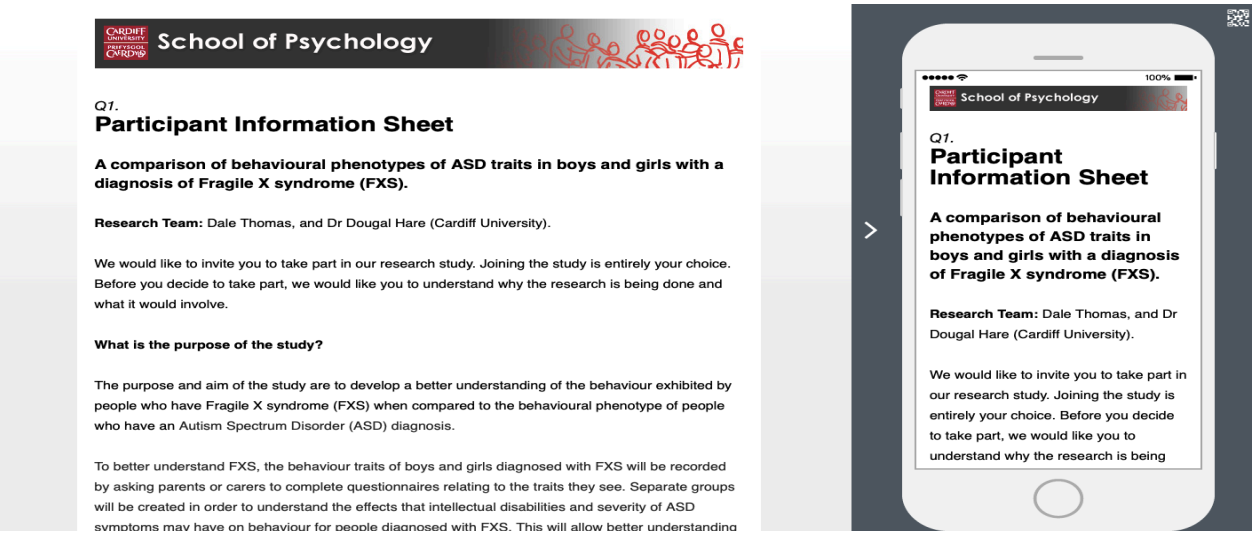
									comparisons relative to chance	
	14/14		2	2	2	2	2	2	2	
23.(Wolff et al., 2012)		Similarities and differences in behavioural expression of autism in FXS and idiopathic Autism (iAut).	Control group group of 38 boys with iAut	23 males with FXS 38 males with iAut	Multiple methods eg. Universities, TEACCH and area clinics	Southern blotting techniques FMRP (FX protein expression)	S/VM (ADOS; RBSR; Mullen)	No developmental trajectory, data collected at one time point.	DS BC	Supports findings that phenotypical heterogeneity of autism and its unique presentation in FXS.
	11/14		2	2	2	2	1	0	2	

Appendix D

Qualtrics

Due to the size, a sample of the first 5 pages of the online questionnaire is provided. Full questionnaire available on request.

A comparison of Behavioural phenotypes of ASD traits in boys and girls diagnosed with Fragile X syndrome.


School of Psychology

Q1.

Participant Information Sheet

A comparison of behavioural phenotypes of ASD traits in boys and girls with a diagnosis of Fragile X syndrome (FXS).

Research Team: Dale Thomas, and Dr Dougal Hare (Cardiff University).

We would like to invite you to take part in our research study. Joining the study is entirely your choice. Before you decide to take part, we would like you to understand why the research is being done and what it would involve.

What is the purpose of the study?

The purpose and aim of the study are to develop a better understanding of the behaviour exhibited by people who have Fragile X syndrome (FXS) when compared to the behavioural phenotype of people who have an Autism Spectrum Disorder (ASD) diagnosis.

To better understand FXS, the behaviour traits of boys and girls diagnosed with FXS will be recorded by asking parents or carers to complete questionnaires relating to the traits they see. Separate groups will be created in order to understand the effects that intellectual disabilities and severity of ASD symptoms may have on behaviour for people diagnosed with FXS. This will allow better understanding

School of Psychology

Q1.

Participant Information Sheet

A comparison of behavioural phenotypes of ASD traits in boys and girls with a diagnosis of Fragile X syndrome (FXS).

Research Team: Dale Thomas, and Dr Dougal Hare (Cardiff University).

We would like to invite you to take part in our research study. Joining the study is entirely your choice. Before you decide to take part, we would like you to understand why the research is being

Survey Flow

Standard: Participants Information
Standard: Consent to Participation
Standard: Demographics
Standard: Needs Assessment
Block: Repetitive Behaviours
Standard: SRS- 2
Standard: Wessex Questionnaire
Standard: Debrief

Page Break

Start of Block: Participants Information

Q1 **Participant Information Sheet.**
A comparison of behavioural phenotypes of ASD traits in boys and girls with a diagnosis of Fragile X syndrome (FXS).

Research Team: Dale Thomas, and Dr Dougal Hare (Cardiff University). We would like to invite you to take part in our research study. Joining the study is entirely your choice. Before you decide to take part, we would like you to understand why the research is being done and what it would involve.

What is the purpose of the study? The purpose and aim of the study are to develop a better understanding of the behaviour exhibited by people who have Fragile X syndrome (FXS) when compared to the behavioural phenotype of people who have an Autism Spectrum Disorder (ASD) diagnosis. To better understand FXS, the behaviour traits of boys and girls diagnosed with FXS will be recorded by asking parents or carers to complete questionnaires relating to the traits they see. Separate groups will be created in order to understand the effects that intellectual disabilities and severity of ASD symptoms may have on behaviour for people diagnosed with FXS. This will allow better understanding of the similarities and differences in behaviour between people who have been diagnosed with FXS when compared to people with an ASD diagnosis. The study also aims to compare the observable characteristics of boys diagnosed with FXS with that of girls diagnosed with FXS. Girls (XX) tend to have a milder presentation of FXS as they have one healthy X chromosome alongside the mutated X chromosome. Boys (XY) do not have the extra unaffected X chromosome. This appears to result in an increased likelihood of intellectual disabilities. Comparing observable traits of boys and girls with FXS will improve our understanding of gender differences and enable us to analyse the impact of variables such as degree of intellectual disability and autistic traits.

Why have I been invited to take part in this study?
You have been invited to take part because you are part of the Fragile X Society research list and have previously given your permission to be contacted to take part in research related to FXS. By being on this list we have assumed that you are a parent of a child with FXS. We are looking for parents whose children are under the age of 16 and have a confirmed diagnosis of FXS who live in the UK.

Do I have to take part?
No, you do not have to take part in the study if you do not want to. Taking part in the research is voluntary; this means it is completely up to you to take part. Your decision to participate in this study will not be connected to the care you and your family are receiving now or in the future. If you decide to take part and sign the consent form but change your mind later, you are free to withdraw at any point and do not need to give us a reason. There will not be any consequences to your current or future treatment if you decide to do this.

Q2 **What will participation involve?**
Parents/ carers will complete a set of questionnaires, which ask about their demographic details, your child's FXS presentation, if you feel you are receiving the right (or enough) support, and if you feel you are receiving the right (or enough) support for your child's education and health needs. Together, these questionnaires will take about 30 minutes to complete. If you are completing paper copies you will be provided with a pre-paid envelope to return the questionnaires.

What are the possible disadvantages and risks of taking part?
It is possible that the questionnaires might raise issues that could be distressing to think about. A list of agencies and people you can contact is provided should you need any additional information/support.

What are the possible benefits of taking part?
The information gained will help services to better understand the needs of a child with FXS and identify ways services can help better meet those needs. This will help clinicians to develop appropriate support packages, which may help other families in the future.

Will my taking part in the study be kept confidential?
Yes. We will handle data sensitively and in confidence and follow legal and ethical guidelines. All data collected about you and your child will be kept strictly confidential and only viewed by members of the research team. It will be stored securely in a locked filing cabinet at the University. Data will be entered onto a computer database which will be password protected and encrypted. Each participant will be assigned a number; thus, names will not be entered onto the database. We plan to publish the research and names of participants will **not** be used. All published data will be anonymous.

What if there is a problem?
It is unlikely that anything would go wrong, but if you have a concern about any aspect of the study, you should contact one of the researchers. If you are not satisfied and wish to make a formal complaint, you can do so through the Cardiff University School Research Ethics Committee complaints procedure. Details can be obtained from the University by calling 029 2087 4000.
In the event that something does go wrong and you are harmed during the study and this is due to somebody's negligence, then you may have grounds for a legal action for compensation against Cardiff University, but you might have to pay your legal costs.

Will I receive any payment for taking part in the study?
Participants will not receive any payment for taking part.

Who is organising the research?
This research is being conducted as part of the Doctorate in Clinical Psychology at Cardiff University for Trainee Clinical Psychologist/postgraduate student Dale Thomas. This study will be carried out under the guidance of Dr Dougal Hare (Academic Supervisor). It is funded by Cardiff University.

Where will the findings be published?
We intend to publish the results in peer-reviewed journals. We intend to present the results at scientific and other relevant conferences. We may put a summary of the findings in the Fragile X Society newsletter. We will provide participants with a summary of the findings if they would like this.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee who protect the rights, safety, dignity and wellbeing of participants. This study has been reviewed and given a favourable opinion by the Cardiff University School of Psychology Research Ethics Committee.

Who can I contact for further information?
If you would like to discuss the study or have any questions or concerns, please do not hesitate to contact Dale Thomas via email on thomasdn@cardiff.ac.uk or tel. 02920 870582. Alternatively, you can contact Dr Dougal Hare, Department of Clinical Psychology, 11th Floor Tower Building, Park Place, Cardiff University, Cardiff CF10 3AT.

End of Block: Participants Information

Start of Block: Consent to Participation

Q3 Please write your name below

Q4 The following questions relate to your consent to participate in this study.

	Yes (1)	No (2)
I confirm that I have read the information sheet for this above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. (1)	○	○
I understand that I can withdraw from the study at any time and have my data removed, without necessarily having to give reasons for this, and that there would not be any adverse consequences of doing so (2)	○	○
I understand that data collected during the study may be looked at by individuals from Cardiff University where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data. (3)	○	○
I understand my participation is anonymous and my confidentiality will be upheld at all time. (4)	○	○
I agree to take part in the above study. (5)	○	○

Q5 Please sign in the box (with your mouse or on touch screen) below to confirm the answers above as your own.

End of Block: Consent to Participation

Start of Block: Demographics

Q6 What is the first part of your postcode (e.g. CF10)?

_____

Q7 What is your relationship to the child you are answering this question about?
(If you have more than one child with FXS please complete two separate surveys, one for each child you wish to include.)

- ○ Parent (1)
- ○ Other family member (2)
- ○ Carer (3)
- ○ Residential/Hostel Staff (4)
- ○ Other (5)

Q8 Is your child:

- ○ a girl (1)
- ○ a boy (2)

Q9 How old is your child?

- ○ 0-5 years old (1)
- ○ 6-10 years old (2)
- ○ 11-15 years old (3)

Q10 Does your child have a diagnosis of Autism Spectrum Condition / Autism?

- ○ Yes (1)
- ○ Currently under/awaiting Assessment (2)
- ○ No (3)

Appendix E

Patient information sheet

Participant Information Sheet

A comparison of behavioural phenotypes of ASD traits in boys and girls with a diagnosis of Fragile X syndrome (FXS).

Research Team: Dale Thomas, and Dr Dougal Hare (Cardiff University).

We would like to invite you to take part in our research study. Joining the study is entirely your choice. Before you decide to take part, we would like you to understand why the research is being done and what it would involve.

What is the purpose of the study?

The purpose and aim of the study are to develop a better understanding of the behaviour exhibited by people who have Fragile X syndrome (FXS) when compared to the behavioural phenotype of people who have an Autism Spectrum Disorder (ASD) diagnosis.

To better understand FXS, the behaviour traits of boys and girls diagnosed with FXS will be recorded by asking parents or carers to complete questionnaires relating to the traits they see. Separate groups will be created in order to understand the effects that intellectual disabilities and severity of ASD symptoms may have on behaviour for people diagnosed with FXS. This will allow better understanding of the similarities and differences in behaviour between people who have been diagnosed with FXS when compared to people with an ASD diagnosis.

The study also aims to compare the observable characteristics of boys diagnosed with FXS with that of girls diagnosed with FXS. Girls (XX) tend to have a milder presentation of FXS as they have one healthy X chromosome alongside the mutated X chromosome. Boys (XY) do not have the extra unaffected X chromosome. This appears to result in an increased likelihood of intellectual disabilities. Comparing observable traits of boys and girls with FXS will improve our understanding of gender differences and enable us to analyse the impact of variables such as degree of intellectual disability and autistic traits.

Why have I been invited to take part in this study?

You have been invited to take part because you are part of the Fragile X Society research list and have previously given your permission to be contacted to take part in research related to FXS. By being on this list we have assumed that you are a parent of a child with FXS. We are looking for parents whose children are under the age of 16 and have a confirmed diagnosis of FXS who live in the UK.

Do I have to take part?

No, you do not have to take part in the study if you do not want to. Taking part in the research is voluntary; this means it is completely up to you to take part. Your decision to participate in this study will not be connected to the care you and your family are receiving now or in the future. If you decide to take part and sign the consent form but change your mind later, you are free to withdraw at any point and do not need to give us a reason. There will not be any consequences to your current or future treatment if you decide to do this.

What will participation involve?

- Parents / carers will complete a set of questionnaires, which ask about their demographic details, your child's FXS presentation, if you feel you are receiving the right (or enough) support, and if you feel you are receiving the right (or enough) support for your child's education and health needs. Together, these questionnaires will take approximately 30 minutes to complete.
- If you are completing paper copies you will be provided with a pre-paid envelope to return the questionnaires.

What are the possible disadvantages and risks of taking part?

It is possible that the questionnaires might raise issues that could be distressing to think about. A list of agencies and people you can contact is provided should you need any additional information/support.

What are the possible benefits of taking part?

The information gained will help services to better understand the needs of a child with FXS and identify ways services can help better meet those needs. This will help clinicians to develop appropriate support packages, which may help other families in the future.

Will my taking part in the study be kept confidential?

Yes. We will handle data sensitively and confidentially and follow legal and ethical guidelines.

- All data collected about you and your child will be kept strictly confidential and only viewed by members of the research team. It will be stored securely in a locked filing cabinet at the University.

- Data will be entered onto a computer database which will be password protected and encrypted. Each participant will be assigned a number; thus, names will not be entered onto the database.

- We plan to publish the research and names of participants will **not** be used. All published data will be anonymous.

What if there is a problem?

It is unlikely that anything would go wrong, but if you have a concern about any aspect of the study, you should contact one of the researchers. If you are not satisfied and wish to make a formal complaint, you can do so through the Cardiff University School Research Ethics Committee complaints procedure. Details can be obtained from the University by calling 029 2087 4000.
In the event that something does go wrong, and you are harmed during the study and this is due to somebody's negligence, then you may have grounds for a legal action for compensation against Cardiff University, but you might have to pay your legal costs.

Will I receive any payment for taking part in the study?

Participants will not receive any payment for taking part.

Who is organising the research?
This research is being conducted by Dale Thomas as part of the Doctorate programme in Clinical Psychology at Cardiff University. This study will be carried out under the guidance of Dr Dougal Hare (Academic Supervisor). It is funded by Cardiff University.

Where will the findings be published?
- We intend to publish the results in peer-reviewed journals.
- We intend to present the results at scientific and other relevant conferences.
- We may put a summary of the findings in the Fragile X Society newsletter.
- We will provide participants with a summary of the findings if they would like this.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee who protect the rights, safety, dignity and wellbeing of participants. This study has been reviewed and given a favourable opinion by the Cardiff University School of Psychology Research Ethics Committee.

Who can I contact for further information?

If you would like to discuss the study or have any questions or concerns, please do not hesitate to contact Dale Thomas at thomasdn@cardiff.ac.uk or tel. 02920 870582. Alternatively, you can contact Dr Dougal Hare, Department of Clinical Psychology, 11th Floor Tower Building, Park Place, Cardiff University, Cardiff CF10 3AT.
You can keep this copy of the information sheet.

Appendix F

Support / Debrief letter

Dear

Thank you for agreeing to participate in the research that set '*A comparison of behavioural phenotypes of ASD traits in boys and girls with a diagnosis of Fragile X syndrome (FXS')*.

This study aimed to better understand what the behavioural presentation (phenotypes) of people with Fragile X Syndrome (FXS) looks like and how that differs from Autistic Spectrum Condition (ASC) by asking you to complete questionnaires relating to the traits that you see. The research study used this data to:

- Assess the severity of Autistic symptoms for people diagnosed with FXS
- Assess the impact of intellectual disabilities on FXS (for those who experience both)
- Compare the behavioural presentation of males diagnosed with FXS with that of females diagnosed with FXS

The findings from this research may help inform ways of better supporting people with FXS and their families in the future, for example, by tailoring psychological interventions to better met their needs.

All the data we collected for this study is confidential, all personal and identifiable information will be kept anonymous and only the researcher and relevant members of the research team can access it.

If you have any questions, queries or require further support please email me at thomasdn@cardiff.ac.uk or phone me on 02920870582. Alternatively, you can contact my supervisor, Dr Dougal Hare on the above telephone number or email address hared@cardiff.ac.uk .

This study relied on your participation and time which is greatly appreciated, thank-you again for your support.

Yours sincerely,

Dale Thomas
Trainee Clinical Psychologist

Appendix G

Consent form

Consent Form

Participant ID: _____

Title of Project: A comparison of behavioural phenotypes of ASD traits in boys and girls with a diagnosis of Fragile X syndrome (FXS).
Name of Researcher: Dale Thomas

Cardiff University in collaboration with the Fragile X Society

		Please tick as appropriate
1	I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected, up until the research data has been analysed.	
3	I understand that data collected during the study may be looked at by individuals from Cardiff University where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data.	
4	I understand my participation is anonymous and my confidentiality will be upheld at all time.	
5	I agree to take part in the above study.	

Name of Participant (Parent): _____

Participant Signature: _____

Date _____

Appendix H

Ethics approval

psychethics

- Dale Thomas;
- Dougal Hare

Dear Dale,

The Ethics Committee has considered your PG project proposal: A comparison of behavioural phenotypes of ASD traits in males and females with a diagnosis of Fragile X syndrome (FXS) (EC.19.01.08.5553).

The project has been approved.

Please note that if any changes are made to the above project then you must notify the Ethics Committee.

Best wishes,
Adam Hammond

School of Psychology Research Ethics Committee

Appendix I

PRISMA checklist

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title page & p6
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	P7
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	P9-11
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	P12
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	PROSPERO Receipt no. 134994
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	P13
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	P13
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	P13
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	P13
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	P14
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Appendix C (Cross & Hare, 2013
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Appendix C (Cross & Hare, 2013)

PRISMA 2009 Checklist

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Qualitative; OR or ES
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Qualitative synthesis

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Table P18
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n/a
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Appendix 1 (Prisma diagram)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table P18
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table P18
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table P18
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Qualitative synthesis p20
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15).	Table P18
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see item 16]).	n/a
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	P28
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	P30
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	P32

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	n/a

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097