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Research article

Genetic determinants of longitudinal behavioural trajectories in rare conditions: The case of fragile X syndrome

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ABSTRACT

Despite being a monogenic condition, individual variability in the phenotypic profile of fragile X syndrome (FXS) is substantial, with behavioural outcomes differing in severity and frequency. Existing studies have revealed that common variation in 5-HTTLPR (serotonin) and COMT (dopamine) single nucleotide polymorphisms (SNPs) is associated with behavioural variation in FXS when measured cross-sectionally. However, the associations between SNPs and longitudinal behavioural trajectories in FXS remain unknown. This study explored relationships between three SNPs, selected a priori (5-HTTLPR, COMT and monoamine oxidase A (MAOA)), and trajectories of clinically relevant behaviours in 42 males with FXS. Autistic characteristics, property destruction, aggression, stereotyped behaviour, self-injury, repetitive behaviour, and mood/interest and pleasure were measured at two time points across three years via a series of standardised informant questionnaires. DNA was extracted from saliva samples and a combination of PCR and TaqMan genotyping was performed for genetic confirmation of FXS, and COMT, 5-HTTLPR and MAOA analyses. Results revealed that males with FXS with AA COMT genotype were less likely to display persistent stereotyped behaviour compared to AG or GG genotypes. Participants with the S/S 5-HTTLPR genotype displayed a steeper decline in repetitive and stereotyped behaviours compared to the L/S or L/L genotypes. Participants with the three-repeat MAOA genotype demonstrated a steeper decline in communication skills over three years compared to those with four repeats. This study documents the association between common genetic variation and behavioural trajectories in males with FXS. Results suggest specific SNPs play an important role in longitudinal behavioural patterns in FXS. This work may facilitate an understanding of individual trajectories for people with FXS, and, therefore, support future tailored interventions.

1. Background

Neurodevelopmental conditions with clear genetic aetiologies provide unique and important insights into gene-behaviour associations. A wealth of research over the last two decades has identified and refined behavioural phenotypes for a multitude of different rare genetic syndromes [51]. More recently, longitudinal studies have been conducted to understand how behaviour changes over time in these populations. A key lesson learned from this body of research is that there is striking variability in behavioural outcomes both across and within genetic syndromes [41]. Whilst this might be anticipated *across* genetic syndromes because of their distinct aetiologies, variability in outcomes *within* syndromes is more intriguing, and currently poorly understood, particularly in the case of monogenic syndromes such as fragile X syndrome (FXS).

Fragile X syndrome (FXS) is a single gene neurodevelopmental condition resulting from a mutation of the fragile X messenger ribonucleoprotein-1 (*FMR1*) gene located on the X chromosome. An expansion of CGG repeats in this gene results in loss of the fragile X messenger ribonucleoprotein (FMRP), an essential protein for cognitive development [3]. Size mosaicism, whereby some CGG repeats are in the normal or premutation range and some CGG repeats are in the full mutation range, and methylation mosaicism, whereby some cells have a methylated FMR1 gene and other cells have an unmethylated FMR1

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gene is observed in FXS [29]. With an estimated prevalence of 1 in 4000 males and 1 in 8000 females [11], FXS is the most common cause of inherited intellectual disability. Alongside intellectual disability, FXS is associated with behavioural and emotional difficulties across the lifespan, including anxiety, aggressive behaviour, hyperactivity, social difficulties, repetitive behaviour and self-injurious behaviour [12]. On average, compared to males, characteristics in females are less severe and their presentations differ qualitatively, likely owing to their second unaffected X chromosome, which results in variable but on average typical amounts of FMRP [1].

Despite the monogenic aetiology of FXS, examples of phenotypic variability are evident in almost any study which reports broader ranges of scores in measures of ability or behaviour than expected from test error. For example, heightened anxiety symptomatology is a hallmark feature of FXS, yet scores on measures of these characteristics span widely from 'normal range' to well above clinical cut-off [16]. Research has sought to identify causal factors that explain variability in phenotypic outcomes. Home-and school-based environmental factors significantly predict the presence of autistic characteristics and internalising and externalising behaviour [23]. Early autonomic arousal predicts later autism diagnoses [43], whilst autistic characteristics predict developmental trajectories of IQ [45], as well as later impulsivity and repetitive behaviour [14]. Methylation mosaicism is associated with better cognitive and behavioural outcomes compared to those with no mosaicism and full methylation [35].

Recently, research has focussed on genetic factors, beyond those implicated in the aetiology of a syndrome, to explain phenotypic variability. Examination of common variation in specific single nucleotide polymorphisms (SNPs) has yielded interesting and clinically important associations with behavioural characteristics. Typically, SNPs associated with variation on a behavioural trait of interest or common complex disease would be identified through genome wide association studies (GWAS). GWAS require sample sizes beyond those achievable in studies of rare diseases given the natural limit in sample size [15,27] and, often in the case of rare diseases, the causative genetic difference is already known, unlike in common complex diseases. An alternative approach, and one that has been adopted in FXS, is to select candidate SNPs, a priori, based on known function, as these effects may moderate the primary effects of FMRP silencing [15,24]. Indeed, evidence suggests many SNPs directly or indirectly affect key neurotransmitters contributing to behavioural regulation and other neural mechanisms [42] that may play a role in moderating phenotypic expression within a syndrome group.

In FXS, there has been a focus on the serotonin-transporter linked SNP (5-HTTLPR), monoamine oxidase A (MAOA) and Catechol-o-methyl transferase (COMT) Val158Met single nucleotide polymorphism (SNP; rs4680) given reports of associations between these genetic factors and behaviours that are commonly reported in FXS (see [15,24]). 5-HTTLPR is a polymorphism in the promotor region of the SLC6A4 gene. This gene codes for the serotonin transporter protein (5-HTT), which facilitates serotonin reuptake from the synapse. Although additional allele subtypes have been identified, two alleles of 5-HTTLPR are commonly investigated in the extant literature: a short (S) variant and a long (L) variant. Compared to the L allele, the S allele is associated with lower 5-HTT expression and function. The genetic variation in MAOA consists of a 30 base-pair variable number tandem repeat (VNTR) located in the promotor region of the MAOA gene that can include 2, 3, 3.5, 4, or 5 copies, with the 3 or 4 repeats most commonly found in Caucasians. The presence of 3.5 or 4 repeats is associated with higher expression, whereas 3 repeats results in lower expression. MAOA is an enzyme that is encoded by the MAOA gene on the X chromosome (Xp11.23), which breaks down neurotransmitters in the monoamine system, including serotonin, dopamine, adrenalin, and noradrenaline, each of which play crucial roles in regulating key behaviours. The COMT gene encodes the COMT enzyme, which breaks down catecholamines, including dopamine, adrenaline, and noradrenaline in the prefrontal regions of the brain. The allelic variation in the Val158Met polymorphism consists of the A (Met) allele and the G (Val) allele. Compared to the Val allele, the Met allele reduces the COMT enzyme activity, which in turn results in lower dopamine-degrading activity and lower prefrontal dopamine receptor density, ultimately resulting in higher dopamine in the prefrontal cortex. COMT genotypes are expressed as either homozygous (AA [MetMet], GG [ValVal]) or heterozygous (ValMet).

Increased levels of stereotyped behaviour (repetition of physical movements and sounds), destructive behaviour and aggressive behaviour have been reported in males with FXS and the long 5-HTTLPR (L/L) allele when compared to those with variants of the short allele (S/S, L/S). Participants homozygous for the short allele (S/S) displayed the least aggressive behaviour. No associations were found between the MAOA genotypes and behavioural expressions in FXS [24]. We recently sought to replicate this work by examining associations between clinically relevant phenotypic behaviour and variation in the 5-HTTLPR and MAOA SNPs, as well as COMT, in males with FXS [15]. Consistent with Hessl et al. [24] no significant associations between MAOA genotypes and phenotypic variation in FXS emerged. However, unlike the study by Hessl and colleagues, there was no significant association between 5-HTTLPR genotypes and behaviour [15]. Interestingly, important associations between behaviour and variation in the COMT genotype were revealed. Specifically, the AA genotype was associated with reduced risk for property destruction, stereotyped behaviour, compulsive behaviour and anhedonia compared to the AG and GG phenotypes. Although these studies provide insight into genetic risk markers associated with the behavioural phenotype of FXS, both studies examine SNPs in relation to behavioural variation cross-sectionally, revealing little about the association between SNPs and longitudinal behavioural patterns in FXS.

As well as within-syndrome variation in behavioural characteristics measured cross-sectionally, individual variation exists in longitudinal developmental trajectories. For example, group level analyses indicate a decline in IQ scores over 24 months for boys aged 3–11 years with FXS but individual trajectories show that whilst some boys demonstrate a striking decline, others show stability, or a slight improvement [10]. A similar diversity in developmental trajectories has been reported for very young children with FXS [21,33]. Research on factors contributing to variability in behavioural trajectories is limited to internal characteristics (i.e. other behaviours such as, for example, attention) and ability levels (see [13,14,17,44]). The extent to which genetic markers contribute to developmental trajectories is currently unknown.

To our knowledge, no other study has examined SNPs in relation to behavioural *trajectories* in FXS. Identifying possible genetic risk markers for behavioural patterns over time in FXS would further understanding of life trajectories enabling improved planning, early intervention programmes, individualised interventions and tailored support. The current study builds upon earlier work by exploring the association between variation within the three SNPs (COMT, MAOA and 5-HTTLPR) with behavioural trajectories in males with FXS across two time points spanning three years. Based upon previous cross-sectional work, we hypothesise a reduction in property destruction, stereotyped and compulsive behaviours, and an increase in interest and pleasure in the environment, over three years in those with the AA COMT genotype, but not in those with AG or GG genotypes. Secondly, we hypothesise that those homozygous for the long 5-HTTLPR allele (L/L) will display increased aggressive, stereotyped and destructive behaviour over time, and those with the S/S genotype will display declining trajectories of aggressive behaviour. Due to limited literature, all other analyses are exploratory in nature.

2. Methods

2.1. Recruitment

This study was conducted as part of a large-scale longitudinal study

exploring behaviour in children and adults with a range of rare genetic syndromes using standardised informant questionnaires. At Time 1 (T_1; 2003–2004) 762 males with FXS were contacted via the Fragile X Society (a UK-based charity) and invited to participate in the research, of which 211 took part (27 %). Three years later (2006–2007) caregivers of participants from T_1 were re-contacted and invited to participate in the follow-up study. Overall, 148 males (70 %) agreed to take part at Time 2 (T_2). Families that took part at any time point were directly contacted in 2015 and asked whether they would be willing to provide a saliva sample to confirm a diagnosis of FXS and to identify the COMT (AA, AG, GG), 5-HTTLPR (S/S, SL, L/L) and MAOA (number of repeats) genotypes. Sixty-four participants returned a saliva sample.

2.2. Participants

Participants with genetic information as well as questionnaire data from T_1 and T_2 were included in the analysis (n = 42). Genetic testing confirmed a diagnosis of FXS in all participants. Due to phenotypic differences between males and females with FXS, only male participants were recruited. Demographic characteristics of participants included in this study are shown in Table 1. Six participants at T_1 and four participants at T_2 were non-verbal thus, for these participants, proportional formulas were used to account for items with language-based subscales.

2.3. Measures

As part of the large-scale study, caregivers of participants completed the informant-report questionnaire measures below:

2.3.1. Demographic questionnaire

A background questionnaire was used to collect information about participants including age, gender, verbal ability (able to speak or sign more than 30 words), mobility (able to walk unaided) and diagnostic status.

2.3.2. Challenging behaviour questionnaire (CBQ)

The CBQ is a binary scaled questionnaire designed to assess the presence or absence of specific behaviours in people with intellectual disability [26]. The CBQ includes four subscales: self-injurious behaviour, physical aggression, property destruction and stereotyped behaviour- all measured over the last month. Kappa coefficients reported for the CBQ demonstrate moderate to very strong interrater reliability [26].

2.3.3. Mood, interest and pleasure questionnaire- short form (MIPQ-S)

The MIPQ-S is used to assess symptoms of low mood and anhedonia in people with intellectual disability, measured across two subscales (*Mood* and *Interest & Pleasure*) [46]. All items are measured over the previous two weeks, with a higher score indicative of higher mood and greater interest and pleasure in the environment. Both interrater reliability and test-retest reliability have been reported as good [46].

2.3.4. Repetitive behaviour questionnaire (RBQ)

The RBQ is designed to measure the presence of repetitive behaviours (such as rituals, cleaning and preference for routine) in participants with intellectual disability [36]. It has five subscales: stereotyped behaviour, compulsive behaviour, repetitive speech, insistence on sameness and restricted preferences. Good validity, test-retest reliability and internal consistency have been reported for the RBQ [36].

Table 1
Participant demographics at Time 1 and Time 2.

Participant characteristics	T_1 (2003/2004)	T_2 (2006/2007)
Mean age (SD) in years	18.07 (10.08)	20.90 (9.76)
Age range in years	6–41	9–42
% Verbal (speak/ sign > 30 words)	85.7 % (36/42)	90.5 % (38/42)
% Mobile (able to walk unaided)	97.6 % (41/42)	100 % (42/42)

2.3.5. The activity questionnaire (TAQ)

The TAQ is used in the assessment of overactivity and impulsivity behaviours in people with intellectual disability [6]. The TAQ includes three subscales: impulsivity, overactivity and impulsive speech. Test-retest and interrater reliability, as well as internal consistency, are reported as robust [6].

2.3.6. Social communication questionnaire (SCQ)

The SCQ is a screening measure used to assess for behaviours associated with autism. The SCQ includes three subscales: social interaction, communication and stereotyped and repetitive behaviour, with higher scores indicative of greater impairment. Sensitivity (0.90) and specificity (0.86) are good for discriminating between autistic and non-autistic cases [7] and test-retest reliability has been reported as good [47].

2.4. Genetic analysis

DNA was extracted from saliva samples and a combination of PCR and TaqMan genotyping was performed for genetic confirmation of fragile X syndrome, COMT analyses, 5-HTTLPR analyses and MAOA analyses. For the full procedure see Crawford et al. [15].

3. Data analysis

Data were analysed for normality using Shapiro-Wilk tests and visual inspection of quantile-quantile (Q-Q) plots. Where data were not normally distributed ($p < .05$) non-parametric tests were used.

Change scores (difference between behaviour reported at T_1 with that reported at T_2) were calculated at full-scale and subscale level for measures providing continuous data (RBQ, MIPQ, TAQ and SCQ). One-way ANOVAs (for normally distributed data) or Kruskal-Wallis tests (for non-normally distributed data) were conducted to assess differences in scores between the genetic subtypes. For 5-HTTLPR scores were compared between those with S/S, S/L and L/L genotypes. For COMT, scores were compared between those with A/A, A/G and G/G genotypes. Where differences emerged, Mann-Whitney U tests and Independent-T tests were used to locate the source of the difference. Only data from participants with either three or four MAOA repeats were included in the analysis (one participant had 5 repeats and was removed from the analyses, and one participant was missing genotype information), thus independent t-tests and Mann-Whitney U tests were used to identify differences in scores between MAOA genotypes.

The CBQ was analysed separately as it generates binary scaled data. For each subscale of the CBQ, behaviour from T_1 was compared to that of T_2. Participants were categorised as either *persistent* (behaviour was present at both time points), *transient* (behaviour was present at only one-time point) or *absent* (behaviour was not present at either time point). Chi-squared analyses were then used to determine whether behavioural trajectories were associated with variation within the COMT, 5-HTTLPR or MAOA genotypes. Where differences emerged, follow up analyses were used to determine the source of the difference.

4. Results

Table 2 presents the mean subscale and full-scale change scores for each questionnaire generating continuous data as a function of COMT, 5-HTTLPR and MAOA genotypes, alongside the frequencies of each genotype. Negative mean change scores indicate a reduction in behaviour between T_1 and T_2 whereas positive scores indicate an increase in the behaviour. Table 3 represents results of the binary scaled CBQ showing the number of participants displaying persistent, transient or absent behaviours between T_1 and T_2.

Table 2
T_2-T_1 mean change scores at sub-scale and full-scale level by SNP genotypes. Bold font indicates a significant difference between subgroups.

	COMT			5-HTTPLR			MAOA repeats	
	GG $n=6$	AG $n=27$	AA $n=9$	L/S $n=17$	S/S $n=10$	L/L $n=15$	3 $n=10$	4 $n=30$
Mood, Interest and Pleasure Questionnaire								
Mood	1.33 (1.03)	0.41 (3.02)	−0.88 (4.67)	−0.94 (4.30)	1.60 (1.71)	0.73 (2.15)	0.11 (2.15)	0.13 (3.49)
Interest & Pleasure	−1.00 (3.41)	−0.57 (4.53)	−2.34 (4.72)	−0.5 (5.34)	−0.85 (3.90)	−1.60 (3.72)	−1.33 (3.50)	−1.12 (4.70)
Total score	0.17 (4.02)	0.02 (6.70)	−3.25 (8.21)	−1.50 (9.19)	0.75 (4.99)	−0.53 (4.37)	−0.89 (3.26)	−0.95 (7.45)
Repetitive Behaviour Questionnaire								
Stereotyped Behaviour	0.00 (1.87)	−1.50 (3.78)	0.00 (3.50)	−1.75 (3.62)	−1.33 (2.55)	0.67 (3.90)	−0.80 (3.26)	−1.14 (3.79)
Compulsive behaviour	0.50 (4.41)	−0.08 (5.95)	0.67 (1.12)	−1.25 (6.88)	−0.10 (3.63)	0.66 (3.42)	0.30 (3.80)	−0.24 (5.32)
Repetitive Speech	1.00 (0.26)	0.26 (4.02)	0.11 (3.48)	−1.00 (4.35)	0.14 (1.86)	1.87 (3.15)	1.14 (3.80)	0.15 (3.76)
Insistence on Sameness	0.33 (1.03)	−0.56 (2.19)	0.50 (1.70)	0.00 (1.51)	1.51 (2.27)	−0.27 (2.34)	−0.20 (0.63)	−0.17 (2.33)
Restrictive Preferences	−1.00 (0.82)	0.86 (3.54)	1.11 (2.49)	0.17 (4.12)	−0.21 (1.22)	1.85 (2.12)	0.44 (3.13)	0.67 (3.24)
Total score	1.80 (7.56)	−0.94 (10.55)	2.00 (4.57)	−1.73 (11.78)	−0.75 (3.37)	2.33 (8.33)	1.20 (6.37)	−0.79 (10.15)
The Activity Questionnaire								
Impulsivity	0.17 (3.76)	−1.41 (6.36)	−1.44 (2.24)	−1.76 (5.51)	−1.60 (2.07)	−0.28 (6.72)	−1.80 (9.23)	−0.81 (3.63)
Overactivity	2.06 (4.92)	−2.69 (7.10)	0.46 (3.88)	−2.93 (8.02)	−0.64 (5.70)	0.01 (4.66)	0.60 (7.34)	−1.70 (6.21)
Impulsive speech	−0.50 (3.70)	−0.60 (2.38)	1.33 (1.50)	−0.80 (2.34)	1.29 (3.30)	0.00 (1.83)	−0.78 (1.79)	0.00 (2.60)
Total score	−0.17 (10.14)	−3.90 (12.01)	1.22 (5.70)	−5.31 (10.89)	−0.35 (7.94)	−0.20 (10.71)	−3.10 (14.46)	−1.57 (9.69)
Social Communication Questionnaire								
Stereotyped & Repetitive Behaviour	−0.33 (1.53)	−0.80 (2.08)	−0.56 (1.81)	**−0.44 (1.36)**	**−2.88 (2.30)**	**0.31 (1.25)**	−0.50 (1.69)	−0.70 (2.09)
Social Interaction	−1.00 (0.82)	−0.43 (0.79)	−0.11 (0.78)	−0.41 (0.87)	−0.33 (0.52)	−0.46 (0.88)	−0.22 (0.66)	−0.56 (0.82)
Communication	−0.61 (0.95)	−0.03 (1.25)	−0.17 (0.99)	0.06 (1.38)	−0.03 (1.20)	−0.42 (0.76)	**−1.12 (0.36)**	**0.09 (1.00)**
Total score	−1.13 (2.78)	−1.00 (3.30)	−0.89 (1.83)	−0.35 (3.06)	−3.83 (3.31)	−0.5 (1.50)	−1.66 (2.55)	−0.86 (3.09)

Table 3
Number showing persistent, transient or absent behaviours across T_1 and T_2, by SNP genotype. Bold font indicates a significant difference between subgroups.

CBQ subscales		COMT			5-HTTLPR			MAOA repeats	
		GG $n=6$	AG $n=27$	AA $n=9$	L/S $n=17$	S/S $n=10$	L/L $n=15$	3 $n=10$	4 $n=30$
Self-Injury	Persistent	3	9	5	7	4	6	5	11
	Transient	1	8	0	6	0	3	1	7
	Absent	2	10	4	4	6	6	4	12
Aggression	Persistent	1	7	2	3	3	4	3	6
	Transient	1	8	2	4	4	3	1	9
	Absent	3	12	4	10	2	7	6	13
Stereotyped Behaviour	Persistent	**4**	**16**	**1**	10	4	7	5	15
	Transient	0	9	5	5	2	7	4	10
	Absent	2	2	3	2	4	1	1	5
Property Destruction	Persistent	1	9	0	4	3	3	1	9
	Transient	0	9	3	7	0	5	3	8
	Absent	5	27	9	6	6	7	6	12

4.1. COMT

Chi-square tests revealed a significant difference between the COMT genotypes in terms of the persistence of stereotyped behaviour, as measured by the CBQ, between T_1 and T_2 (X^2 (4, $N=42$) $=10.74$, $p=0.03$). Post-hoc analyses were conducted to locate the source of this difference and following adjusted residuals and Bonferroni corrections, the corrected level for statistical significance was.005. Although no follow up analyses reached this significance level, a close to significant result emerged: participants with the AA COMT genotype were less likely to display persistent stereotyped behaviours than those with the AG or GG genotypes ($p=.009$) (Fig. 1). This result was close to the corrected significance level and would reach significance at the conventional level of.05. Given the challenges of small sample sizes and limited statistical power in rare syndrome research, this result is important to include. No other differences in scores were revealed between the COMT genotypes.

4.2. 5-HTTLPR

One way ANOVA tests revealed that the mean stereotyped and repetitive behaviour change score over three years, measured by the SCQ, differed significantly between the three 5-HTTLPR genotypes, ($F(2, 34) = 10.61, p < .001$). The L/L genotype group displayed an increase in this behaviour between T_1 and T_2, whereas the S/S and L/S genotypes displayed a reduction in these behaviours over time (see Fig. 2). Post-Hoc Tukey HSD tests revealed that the source of the significant difference was a steeper reduction in repetitive and stereotyped behaviours over time in participants with the S/S genotype compared to both the L/S genotype ($p=.003$) and the L/L genotype ($p<.001$). There was no difference in behavioural trajectories between those with the L/S and L/L genotypes ($p>.05$). No other significant associations were found for any other sub-scale or full-scale item ($p>.05$).

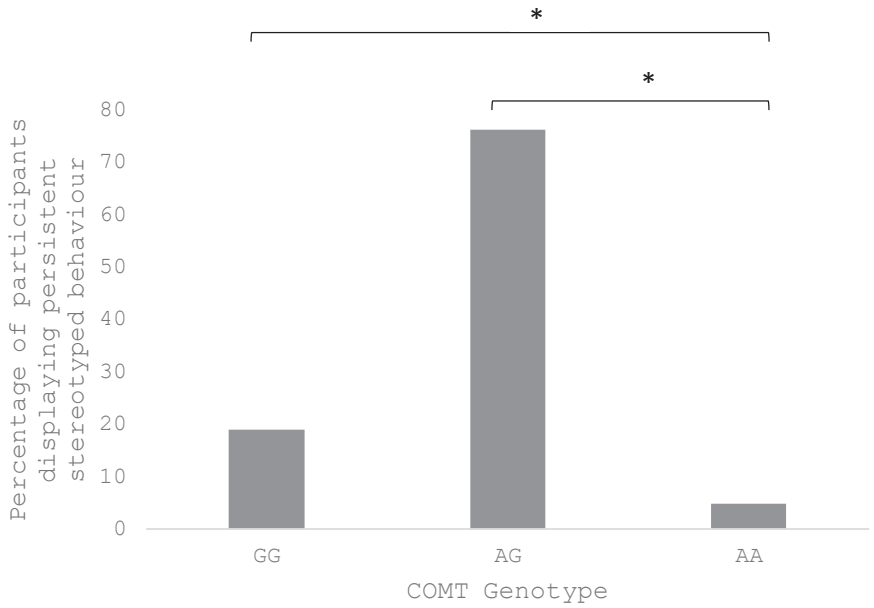

Fig. 1. Percentage of participants displaying persistent stereotyped behaviour, as measured by the CBQ (total $n = 21$) for each COMT genotype.

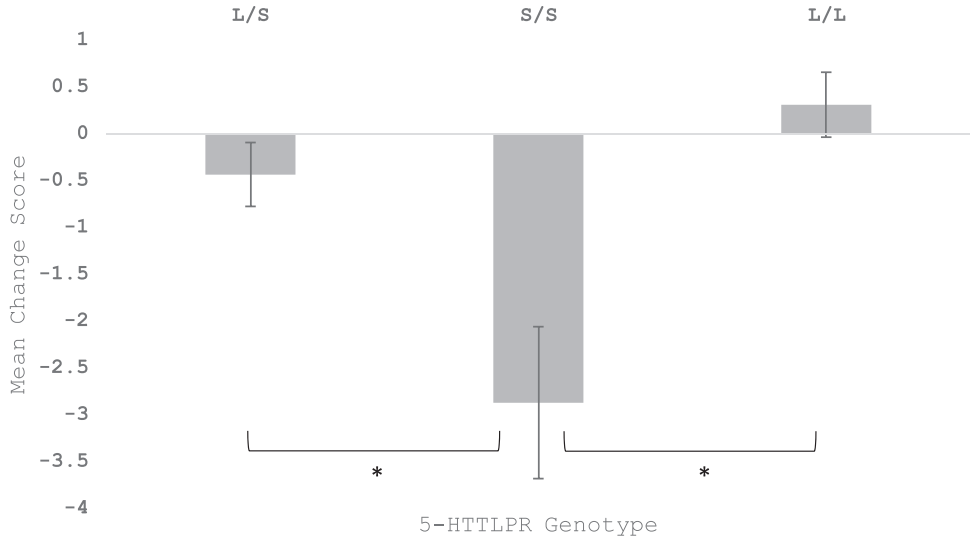

Fig. 2. Mean T_2-T_1 change scores on the SCQ stereotyped behaviour subscale for each 5-HTTLPR genotype (total $n = 42$).

4.3. MAOA

Analyses of behavioural trajectories and MAOA genotypes revealed the three-repeat group displayed a significantly steeper reduction in communication impairment (a subscale of the SCQ) than those with 4 repeats (U=26.50, $p = 0.001$) between T_1 and T_2 (Fig. 3). Communication impairment decreased over time in the three-repeat group, whilst communication impairment increased slightly in the four-repeat group. No other significant associations were found for any other sub-scale or full-scale item ($p > .05$).

5. Discussion

This study builds upon previous research conducted by Crawford et al. [15] by exploring SNPs and longitudinal behavioural patterns in FXS. Common genetic variation in three SNPs (COMT, 5-HTTLPR, MAOA) was analysed alongside the trajectories of phenotypic behaviours, as determined by standardised measures designed for people with intellectual disability, in males with FXS across three years. Analyses of genotype-phenotype associations revealed three key findings: 1) a steeper decline in repetitive and stereotyped behaviour was evident in those homozygous for the S/S 5-HTTLPR genotype compared to S/L or L/L alleles, 2) the three-repeat MAOA group displayed a greater reduction in communication impairments over time than those in the four-repeat group, 3) participants homozygous for the AA COMT genotype were less likely to display persistent stereotyped behaviour over time than those with the GG or AG genotype. Although the third result narrowly missed statistical significance ($p = .009$; adjusted $\alpha = .005$) following Bonferroni corrections, it is important to consider given its possible clinical significance in light of the small sample sizes, and thus reduced statistical power, in rare genetic syndromes.

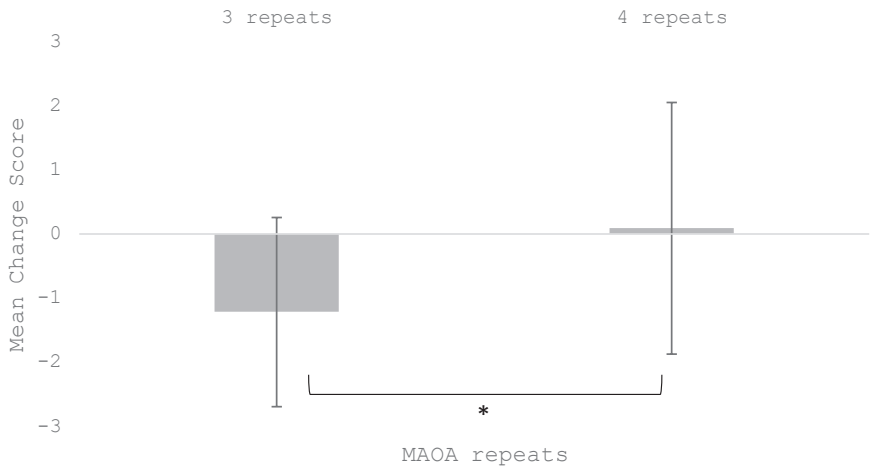

Fig. 3. Mean T_2-T_1 change scores on the SCQ communication subscale as a function of MAOA repeats (total $n = 40$).

5.1. COMT

In our earlier work, the AA COMT genotype was associated with reduced stereotyped behaviours at one time point [15], and the present study found less likelihood of *persistent* stereotyped behaviours over three years in those with the AA genotype compared to the GG or AG genotypes. Together, these results indicate a protective COMT-related genotype (AA) in FXS, associated with fewer stereotyped behaviours both at one time point, and over time.

The COMT gene is responsible for managing catechol-O-methyltransferase, an enzyme important for the breakdown of dopamine [34]. The A allele of the COMT SNP is associated with decreased enzymatic activity, effectively increasing dopamine levels. Therefore dopamine levels will be highest in the AA group and lowest in the GG group [25,48]. Increased dopamine levels have been associated with stereotypic-like behaviours including increased movements and repetitive behaviours like sniffing, biting and licking [30] as well as involuntary hyperkinetic movements in Huntington's (a disease characterised by abnormally high dopamine levels) [28,39]. Arguably, these involuntary motor functions caused by increased dopamine offer much overlap with stereotypical behaviours, e.g. tapping or patting parts of the body, continuous hand movements or eye pressing, which are items indicative of stereotypy [26]. Interestingly, the results from both this and Crawford et al., [15] find the opposite, with reduced stereotyped behaviours in those with the highest dopamine levels (AA). In the case of FXS, for which downstream cascading effects on dopamine function have been reported [53], increased dopamine availability in the AA group might be beneficial in controlling stereotypies. This has been reported in individuals with attention-deficit-hyperactivity-disorder (ADHD) [4,19] indicating that FXS might approximate a hypodopaminergic state (as in ADHD) rather than a hyperdopaminergic state (as in Huntington's disease).

5.2. 5-HTTLPR

Genotype-phenotype analyses of the 5-HTTLPR SNP revealed results consistent with prior FXS literature. Hessl et al. [24] found increased stereotyped, destructive and aggressive behaviours in those homogenous for the L/L 5-HTTLPR genotype cross-sectionally, with least aggression reported in the S/S group. In line with this, in the current study, participants homozygous for the S/S genotype displayed a steeper decline in stereotyped and repetitive behaviours over time than both other genotypes. These results indicate a favourable phenotypic profile in FXS for those homozygous for the short allele, whilst the L/L allele appears to be associated with less favourable behavioural outcomes, particularly an increased and persistent expression of stereotyped behaviours.

Compared to the long allele, the short allele is associated with reduced transcriptional efficiency, and expression, of the serotonin transporter [52]. This is thought to influence multiple systems impacting stress response and reactivity, emotional regulation, fear processing in the amygdala and hypothalamic-pituitary-adrenal (HPA) axis function [49]. There is a plethora of research documenting impairment in these systems in FXS [32] and so an association between 5-HTTLPR and behaviour in this population is unsurprising. What is interesting is that the link between 5-HTTLPR and externalising behaviour in the general population is the opposite to that reported in FXS. Whilst in FXS the S/S genotype is associated with more favourable outcomes, the majority of reports in the general population indicate an increased susceptibility for negative social and/or emotional outcomes in individuals with at least one copy of the S-allele [8,18,40]. This reversed genotype-phenotype relationship relative to typical individuals may be related to the differing genetic and/or environmental landscapes associated with neurodevelopmental conditions like FXS. Few longitudinal studies exploring 5-HTTLPR genotype and behaviour exist but one recent study indicates an important gene X environment association in the development of anti-social behaviour in the general population [49].

5.3. MAOA

Contrary to the findings reported here, the majority of cross-sectional reports indicate the 3-repeat MAOA allele as being associated with more negative outcomes. A meta-analysis revealed significant associations between the 3-repeat allele and anti-social behaviour [20]. In addition, boys with the 3-repeat allele had more severe sensory behaviours, arousal regulation problems, aggressive behaviour and poorer communication skills than autistic males with the 4-repeat allele [9]. As with the other SNPs involved in the current study, limited longitudinal studies exist. Although most existing research appears divergent with our findings, some prior research within other populations has implicated the four-repeat MAOA allele with an increased risk of aggressive behaviour when compared to the three-repeat allele [2,31]. With a well-established link between communication deficits and aggression in intellectual disability [22,38], our results support a possible connection between these behaviours.

The findings reported here regarding both 5-HTTLPR and MAOA are divergent from the majority of those reported in the general population. Although not completely unanimous, most studies indicate that the genetic variants that result in increased availability of synaptic serotonin are associated with increased risk for poor outcomes. Whilst this might be indicative of different genetic landscapes in neurodevelopmental conditions compared to the general population, it might also be due to studies of the general population measuring qualitatively different

behaviours than in neurodevelopmental conditions (e.g. anti-social behaviour in the general population and aggressive behaviour in neurodevelopmental conditions). In turn, this may lead to over-simplification of conclusions regarding positive and poor outcomes. In addition, there is a dearth of longitudinal SNP X phenotype studies, even in the general population. Therefore, attempting to link findings from our longitudinal study to existing cross-sectional studies may not be possible due to a lack of comparability. The current study informs future research by highlighting the importance of studying behaviours at a fine-grained level, This is evidenced by the identification of genotype-phenotype associations with some behaviours and not others. The study also showcases the merit of studying behavioural trajectories longitudinally to more fully understand associations with genotypes that were not present when exploring associations with the same participants cross-sectionally. Identifying potential genetic causal factors contributing to the phenotypic heterogeneity in FXS has implications for precision medicine-based approaches. It has been proposed that clinical trials may be more successful if underlying heterogeneity in FXS is considered by stratifying subpopulations of drug-responders [50]. A future line of research could identify if the genetic markers of heterogeneity identified here map onto drug responder profiles. Another next step for this line of research is to begin exploring gene X environment associations and the role they play in the development and maintenance of behaviour. This is important in FXS given that sensitivities to environmental influences on behaviour have been identified in this population [23]. Additionally, the role of SNPs alongside other genetic factors known to contribute to phenotypic variability, such as methylation mosaicism, is important to consider.

Overall, the results should be considered in light of some weaknesses. First, as is often the case in research with rare syndromes, the sample size is a limitation. Although, overall, this is comparable to other research within rare conditions, the distribution of participants by genotype created subgroups, which may explain why post-hoc analyses did not reach significance. In addition, the current study did not include IQ measures to characterise the ability level of participants. However, this was a product of conducting a postal survey in order to maximise the response rate for the largest possible sample size. Finally, the questionnaire data were collected a long time ago and may not fully represent societal and interventional advances that have occurred since. Nevertheless, there are several strengths to this study. Three SNPs were examined alongside numerous clinically relevant behaviours in FXS using a number of standardised measures, that have each been designed specifically for people with intellectual disability, to assess a wide range of behavioural characteristics. Moreover, the longitudinal design of this study enabled exploration of behavioural trajectories in FXS in relation to SNPs. This study highlights the merit of investigating SNPs, that are selected a priori in terms of their mechanisms of action, in rare populations. This approach is complex and findings do not always replicate. For example, a multiple hit hypothesis has been proposed in the context of other genetic syndromes ([5] for sex chromosomal trisomies) but later data did not support the hypothesis [5]. Other multiple hit accounts, involving copy number variants (CNVs), rather than SNPs, have also been proposed and tested [37]. As a whole, it is therefore an approach that has been leveraged beyond FXS, but further and larger replication studies are very obviously needed. However, these studies still require large sample sizes, relative to the availability of participants with such rare conditions. As with genotype phenotype studies conducted in the general population, achieving the ideal sample size for genotype phenotype studies in rare populations is more likely through multi-site collaboration.

6. Conclusions

This study explored the association between SNPs and behavioural trajectories in FXS, with results highlighting an important role of each SNP in longitudinal patterns of behaviour over three years. The research presented furthers our understanding of the genetic markers that contribute to varied behavioural trajectories in FXS, which may support future tailored interventions.

Ethics approval

Ethical approval for this study was obtained by the NHS Coventry Research Ethics Committee (23.09.2015/Ref: 10/H1210/1).

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CRediT authorship contribution statement

Stockton Joanne: Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Wilde Lucy:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Oliver Chris:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **Beggs Andrew:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Cartwright Lydia:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Scerif Gaia:** Writing – review & editing. **Crawford Hayley:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

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Data statement

The datasets generated and analysed during the current study are not publicly available. Due to the sensitive nature of the research and ethical concerns surrounding publication of sensitive personal data, no participants were asked for consent to their data being shared.

Consent to participate

Informed consent was obtained from all individual participants included in the study where possible or by consent by proxy in accordance with ethical guidelines.

Data availability

The data that has been used is confidential.

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