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Association Between Macroorchidism and Intelligence in *FMR1* Premutation Carriers

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Abstract

Characteristics of fragile X syndrome include macroorchidism and intellectual disability, which are associated with decreased FMRP levels. FMRP is highly expressed in many tissues, but primarily in the brain and testis. The relationship between these two characteristics has not previously been studied in the premutation or carrier state. To examine this among premutation carriers and a possible association with IQ, we evaluated macroorchidism status among 213 males including 142 premutation carriers and 71 controls. The prevalence of macroorchidism among premutation carriers was 32.4% (46 out of 142), and 5.6% among controls (4 out of 71, P <0.0001). Among premutation carriers, the age-adjusted odds ratio (OR) of macroorchidism was significantly increased with increasing *FMR1* mRNA (OR 1.84, 95% confidence interval [CI] 1.04–3.25; P 0.035). With respect to the association between macroorchidism and IQ, after adjustment for number of CGG repeats and age, premutation carriers with macroorchidism had lower verbal IQ (104.67±15.86, P 0.0152) and full scale IQ (102.98±15.78, P 0.0227) than premutation carriers without macroorchidism (verbal IQ 112.38 ±14.14, full scale IQ 110.24±14.21). Similar associations were observed for both verbal IQ (P 0.034) and full scale IQ (P 0.039) after being adjusted for age and *FMR1* mRNA. These preliminary data support a correlation between macroorchidism and lower verbal and full scale IQ in a relevant proportion of premutation carrier males. Whether this is due to higher levels of FMR1 mRNA or to lower FMRP levels it remains to be established.

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Keywords

premutation; *FMR1* gene; premutation; macroorchidism and biomarker

INTRODUCTION

Fragile X syndrome (FXS) and Fragile X-associated disorders (FADs) are related to mutations in the Fragile X Mental Retardation 1 gene (*FMR1*). In the normal range, there are 5 to 44 CGG repeats in the 5′ untranslated region of *FMR1*. The Fragile X mutation occurs in two forms, the premutation with 55–200 CGG repeats and the full mutation with more than 200 repeats. There is also a gray zone for individuals with 45–54 CGG repeats who may or may not present with premutation manifestations [Loesch et. al., 2011; Sullivan et. al., 2011]. The prevalence of the full mutation is approximately 1 in 4000 males and 1 in 8000 females [Coffee et. al., 2009; Crawford and et. al.,2001]. The average IQ of an adult male with FXS is approximately 40, but those with a lack or partial lack of methylation (methylation mosaicism) or those who have size mosaicism have an average IQ in the 60s [Merenstein et. al., 1996]. Approximately 15% are high functioning with an IQ greater than 70. Most girls with FXS, on the other hand, have an IQ above 70, although 25% have intellectual disability. The X-activation ratio, meaning the percentage of cells with the normal X chromosome active, correlates with the overall IQ in girls with FXS [Hagerman and Hagerman, 2002].

In the full mutation, or occasionally the higher end of the premutation range, methylation prevents transcription of FMR1 mRNA and the consequent *FMR1* protein (FMRP) deficit results in intellectual disability and other physical features seen in FXS. Conversely, the more typical finding in the premutation range is very high levels of *FMR1* mRNA leading to RNA toxicity [Hagerman, 2013; Tassone et. al., 2000]. The number of CGG repeats has been shown to directly correlate with the amount of FMR1 mRNA [Ludwig et. al., 2014]. Clinically, this RNA toxicity can lead to psychiatric difficulties in childhood including attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (ASD), shyness and social anxiety [Chonchaiya et. al., 2012]. ADHD and ASD can persist into adulthood, but depression and anxiety are the most common psychiatric disorders of adults with the premutation [Bourgeois et. al.,2011].

The premutation is also the most common genetic cause of primary ovarian insufficiency, defined as cessation of menses before age 40, and occurs in approximately 20% of female carriers [Sullivan et. al., 2011]. Neurological problems are common in adults with the premutation including neuropathy, tremor, ataxia and cognitive decline; these are the primary symptoms of the fragile X-associated tremor ataxia syndrome (FXTAS). The FXTAS incidence is approximately 40% of male carriers and 16% of female carriers. Females carriers with FXTAS have milder symptoms than males and rarely have dementia [Hagerman, 2013]. The prevalence of the premutation in the general population is approximately 1 in 200 females and 1 in 450 males [Tassone et. al., 2012].

In children with the premutation, IQ scores were found to be lower than in controls, but not to a statistically significant degree [Myers et. al., 2001]. However, children and young adults

with the premutation have a higher incidence of ADHD and showed executive function deficits [Cornish et. al., 2009; Grigsby et. al., 2008; Moore et. al., 2004a; Moore et. al., 2004b], which can impact cognitive function and IQ scores.

FMR1 is highly expressed in the brain and testis in the normal population and the RNA toxicity of FXTAS is associated with the formation of inclusions in neurons [Leehey et. al., 2008; Tassone et. al., 2007] and testicular Leydig cells in carriers [Gokden et. al.,2009; Greco et. al., 2007; Hunsaker et. al., 2011]. Macroorchidism is observed in almost all individuals with FXS and anecdotally in premutation carriers. The present study specifically characterizes the frequency of macroorchidism in premutation carriers and its association with cognitive abilities and molecular measures.

MATERIALS AND METHODS

Data Collection

Data relevant to this study were collected from 218 male research subjects with Tanner Stage IV pubertal development; macroorchidism was defined as a testicular volume >30 mls. This included 46 premutation carriers with macroorchidism and a mean age of 56.35 years, 96 carriers without macroorchidism with a mean age of 57.39 years and 67 control subjects with a mean age 45.36 years. Each subject had been actively recruited to the UC Davis MIND Institute for participation in studies that all required a medical history and physical examination. All subjects signed an informed consent document as part of IRB project approval. Each physical exam sheet completed by a physician indicated the presence or absence of macroorchidism in the subject. Most subjects (70% of controls and 98% of premutation carriers) also had a quantitative bilateral measurement of testicular volume recorded. The average testicular volume after puberty is 18 cm^3 (ml) with a normal size ranging from 12 cm^3 to 24 cm^3 [Goodman and Gorlin, 1983; Meschede et. al., 1995; Zachmann et. al., 1974]. Macroorchidism was defined as a testicular volume greater than 30$cm^{3.}$ Testicular volume was measured by a Prader orchidometer, which in previous correlates well with the gold standard of ultrasound measurements [Paltiel et. al., 2002]. All subjects included in this study also completed the Wechsler Adult Intelligence Scale-IV or III [Wechsler 1997].

Molecular Status

Molecular measure of the CGG trinucleotide expansion was used to separate controls from premutation carriers. Analysis of blood drawn from research subjects was completed using an Alpha Innotech FluorChem 8800 Image Detection System (Alpha Innotech Co., San Leandro, CA). The specific protocol has been previously outlined [Saluto et. al., 2005; Tassone et. al., 2000; Tassone et. al., 2004]. Repeat sizes between 55 and 200 inclusive were considered premutation carriers. Repeat numbers under 45 were treated as controls. Subjects in the full mutation range (above 200 CGG repeats) or in the gray zone (45–55 repeats) were excluded from this study. Subjects displaying mosaicism, regardless of CGG repeat number, were also excluded. Serum *FMR1* mRNA levels were collected and examined when available in a method described previously [Tassone et. al., 2000].

Statistical Analysis

Descriptive statistical analysis was based on Chi-square test for categorical variables and the analysis of variance (ANOVA) for continuous variables. Logistic regression was used to compare the age-adjusted odds ratio of macroorchidism status among premutation carriers, as a function of molecular variants, CGG and *FMR1* mRNA. Comparisons of verbal IQ, performance IQ, and full scale IQ across three groups (premutation carriers with macroorchidism, premutation carriers without macroorchidism and controls without macroorchidism) were performed using the analysis of covariance (ANCOVA). All statistical analysis was conducted in SAS version 9.2.

RESULTS

Characteristics of study subjects

The initial cohort (N=213) comprised 142 premutation carriers and 71 controls. The prevalence of macroorchidism among premutation carriers was 32.4% (46 out of 142) and 5.6% among controls (4 out of 71, P <0.0001). After excluding the four controls with macroorchidism, the final analysis cohort (N=209) included 46 premutation subjects with macroorchidism (group A); 96 premutation subjects without macroorchidism (group B); and 67 control subjects without macroorchidism (group C). Table I summarizes the study participant characteristics and unadjusted descriptive analysis results across three groups. Among premutation carriers, age and CGG showed no significant difference with regard to macroorchidism status (A vs. B), but *FMR1* mRNA in premutation carriers with macroorchidism (Mean 3.24, SD1.26) was significantly higher than premutation carriers without macroorchidism (Mean 2.76, SD 0.82, P 0.0021). Verbal IQ, performance IQ, and full scale IQ of both group A and group B were significantly different from group C (<. 0001).

Macroorchidism in premutation carriers and association with FMR1 mRNA and number of CGG repeats

The likelihood (odds) of macroorchidism, adjusted for age, was significantly associated with *FMR1* mRNA; given 1 unit increase in *FMR1* mRNA (i.e., about 0.9 SD), the odds ratio of macroorchidism was 1.84 (95% confidence interval [CI] 1.04–3.25; P 0.035). The age-adjusted odds ratio of macroorchidism was not associated with CGG repeat number (Table II).

Associated between macroorchidism and IQ

Table IIIa summarizes Verbal IQ, Performance IQ, and Full scale IQ across three groups, adjusted for age and number of CGG repeats. Verbal IQ measurements among premutation carriers with macroorchidism (Group A: Mean 104.67, SD 15.86) were lower than premutation carriers without macroorchidism (Group B: Mean 112.38, SD 14.14, P 0.0152). Full Scale IQ measurements among premutation carriers with macroorchidism (Group A: Mean 102.98, SD 15.78) were also lower than premutation carriers without macroorchidism (Group B: Mean 110.24, SD 14.21, P 0.0227).

In Table IIIb, after adjusting for age and *FMR1* mRNA levels, both verbal IQ (P 0.034) and full scale IQ (P 0.0399) among premutation carriers with macroorchidism (Group A) was lower than premutation carriers without macroorchidism (Group B). Premutation carriers with macroorchidism (Group A) also had lower full scale IQ (P 0.0363) than controls without macroorchidism (Group C). The results were not statistically significant at level 0.05 after Bonferroni adjustment for multiple comparisons (Table IIIb).

DISCUSSION

Testicular examination can be important in the diagnosis of genetic conditions. There are over 20 genetic syndromes associated with variations in testicular size including Clark-Baraitser syndrome[Mendicino et. al., 2005], aspartylglucosaminuria [Arvio and Arvio 2002], Atkin-Flaitz syndrome[Atkin et. al., 1985], McCune-Albright syndrome[Wasniewska et. al., 2006] and X-linked intellectual disability syndromes. According to the Online Mendelian Inheritance in Man database (omim.org), about two thirds of syndromes associated with macroorchidism are also associated with intellectual disability and roughly one third have an X-linked inheritance pattern. Testicular volume differences can help clarify diagnoses. For example, testicular volume is used to separate Marfan and Klinefelter syndromes. Individuals with both present with grossly similar phenotypes including a tall and thin body habitus, but a hallmark of Klinefelter syndrome is reduced testicular size, whereas individuals with Marfan syndrome typically do not have this finding [Marcell and Ellen 2012].

On the other side of the size spectrum, individuals with FXS often present with signs of a connective tissue disorder including joint laxity [Davids et. al., 1990], mitral valve prolapse and aortic dilation [Sreeram et. al., 1989] similar to individuals with Marfan syndrome. However, it is well known that most of the patients with FXS actually display enlarged testicular volume among other associated signs and symptoms that can help separate it diagnostically [Butler et. al., 1993; Crabbe et. al., 1993]. One of the new findings in this study is that differential diagnosis of macroorchidism should potentially be expanded from FXS to also include the premutation form of the *FMR1* gene. The relative risk of macroorchidism in the premutation population was 5.75 times that of control (5.6%; P<0.001) and was seen in nearly a third of carriers. This could be very useful as the clinical diagnosis of a premutation male carrier is often challenging because most do not present with the typical features of FXS. Early diagnosis of premutation carriers can help patients begin to screen for and treat some of the associated features, such as, anxiety, hypertension, hypothyroidism and migraines which in turn may help to decrease the changes of developing FXTAS [Hagerman and Hagerman 2013].

It is known that the IQ is lower in premutation carriers when compared with the general population[Fisch 2006], however, our study adds that among premutation carriers, macroorchidism was significantly associated with lower verbal IQ after adjustment for age and number of CGG repeats (Supplementary Table Ia, Supplementary Figure 1 in supporting information online). The verbal distinction seen here may relate to some of the verbal deficits seen in FXS, particularly those suffering from autism. Individuals with the premutation and macroorchidism had significantly higher levels of *FMR1* mRNA (OR 1.84,

95% CI 1.04–3.25; P 0.035), but macroorchidism was not strongly associated with number of CGG repeats. While in the full mutation *FMR1* mRNA is decreased and FMRP is absent or significantly decreased, in the premutation the elevated *FMR1* mRNA level is associated with lower levels of FMRP. Often FMRP is also lower in those with a higher number of CGG repeats at the upper end of the premutation range [Brouwer et. al., 2009; Pretto et. al., 2014]. This suggests that higher levels of mRNA may be associated with FMRP deficits in premutation carriers, which could be the cause of both the lower IQ and macroorchidism seen here.

We were not able to carry out FMRP studies in this investigation, however new techniques are being developed to more easily study FMRP levels [LaFauci et. al., 2013; Schutzius et. al., 2013]. There is significant variability of FMRP levels even in the general population [Iwahashi et. al., 2009] and the level of FMRP has been recently associated with cognitive abilities and frontal dysfunction in the general population [Wang et. al., 2013]. This will likely be an area for future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table I

Descriptive statistics of study cohort

Variable	Group A Premutation with macroorchidism			Group B Premutation without macroorchidism			Group C Control without macroorchidism			P-value
	N	Mean	SD	N	Mean	SD	N	Mean	SD	
Age	46	56.35	18.6	96	57.39	16.58	67	45.36	17.54	0.0004
# of CGG Repeats	46	97	25	96	91	23	67	28	5	<.0001
FMR1 mRNA	43	3.24	1.26	94	2.76	0.82	56	1.36	0.28	<.0001
Left Testicular Volume (ml)	46	41.52	10.01	96	23.13	6.36	47	25.47	7.2	<.0001
Right Testicular Volume (ml)	46	41.09	10.28	96	23.25	6.3	46	25.59	7.08	<.0001
Average Testicular Volume (ml)	46	41.3	10.09	96	23.19	6.24	47	25.41	7.17	<.0001
Verbal IQ	46	104.67	15.86	96	112.38	14.14	67	118.99	18.23	<.0001 *
Performance IQ	46	100.46	15.64	96	105.72	15.08	67	115.4	16.93	<.0001*
Full Scale IQ	46	102.98	15.78	96	110.24	14.21	67	119.13	18.3	<.0001*

* For verbal IQ, performance IQ, and full scale IQ, after Bonferroni adjustment, both group A and group B were significantly different from group C

Table II

Association between macroorchidism and *FMR1* mRNA among premutation carriers

Variable	Odds Ratio	95% Confidence Interval		P-value
		Lower	Upper	
AGE	1.00	0.98	1.02	0.8318
# of CGG Repeats	0.99	0.97	1.02	0.4878
FMR1 mRNA	1.84	1.04	3.25	0.035

* Macroorchidism status=No as reference

Table IIIa

Asscoication between macroorchidism and IQ, adjusted for age and CGG repeats

Variable	Least Square Mean ± SE*			P-value		
	Group=A	Group =B	Group =C	Group	Age	# of CGG Repeats
Verbal IQ	108.38 ± 2.7	115.32 ± 1.94	112.21 ± 3.16	0.0481	0.3197	0.01
Performance IQ	104.7 ± 2.64	109.26 ± 1.9	107.41 ± 3.09	0.2545	0.0078	0.0052
Full scale IQ	107.23 ± 2.68	113.7 ± 1.93	111.26 ± 3.14	0.0712	0.0616	0.0042

* SE: Standard error

** Bonferroni adjusted α for entire table = 0.05/9 ~0.0056

Table IIIb

Association between macroorchidism and IQ, adjusted for age and *FMR1* mRNA

Variable	Least Square Mean ± SE			P-value			P-value**		
	Group=A	Group =B	Group =C	Group	Age	*FMR1*mRNA	A vs B	A vs C	B vs C
Verbal IQ	107.45 ± 2.66	113.83 ± 1.73	115.45 ± 2.75	0.0789	0.3409	0.0181	0.034	0.0638	0.6469
Performance IQ	102.99 ± 2.63	107.61 ± 1.71	111.17 ± 2.72	0.1372	0.015	0.0277	0.1198	0.0553	0.3083
Full scale IQ	105.89 ± 2.65	112.05 ± 1.72	114.91 ± 2.74	0.0659	0.0785	0.012	0.0399	0.0363	0.4181

** Bonferroni adjusted α for entire table = 0.05/9 ~0.0056