

FMR1 allele size distribution in 35,000 males and females: a comparison of developmental delay and general population cohorts

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Purpose: Developmental delay phenotypes have been associated with *FMR1* premutation (PM: 55–200 CGG repeats) and “gray zone” (GZ: 45–54 CGG repeats) alleles. However, these associations have not been confirmed by larger studies to be useful in pediatric diagnostic or screening settings.

Methods: This study determined the prevalence of PM and GZ alleles in two independent cohorts of 19,076 pediatric referrals to developmental delay diagnostic testing through Victorian Clinical Genetics Service (cohort 1: $N = 10,235$; cohort 2: $N = 8841$), compared with two independent general population cohorts (newborn screening $N = 1997$; carrier screening by the Victorian Clinical Genetics Service prepair program $N = 14,249$).

Results: PM and GZ prevalence rates were not significantly increased ($p > 0.05$) in either developmental delay cohort (male PM: 0.12–0.22%; female PM: 0.26–0.33%; male GZ: 0.68–0.69%;

female GZ: 1.59–2.13%) compared with general population cohorts (male PM: 0.20%; female PM: 0.27–0.82%; male GZ: 0.79%; female GZ: 1.43–2.51%). Furthermore, CGG size distributions were comparable across datasets, with each having a modal value of 29 or 30 and ~1/3 females and ~1/5 males having at least one allele with ≤ 26 CGG repeats.

Conclusion: These data do not support the causative link between PM and GZ expansions and developmental-delay phenotypes in pediatric settings.

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Key Words: Developmental delay (DD); fragile X mental retardation 1 gene (*FMR1* gene); fragile X syndrome (FXS); premutation; prevalence

INTRODUCTION

Neurological and neurodevelopmental disorders are associated with a trinucleotide CGG expansion of the *FMR1* gene located on the long arm of the X chromosome.¹ Full mutation (FM) CGG expansions of > 200 repeats cause fragile X syndrome (FXS), whereby gene silencing leads to little or no production of the *FMR1* protein (FMRP) that is essential for typical neurodevelopment.² FXS is a common single-gene cause of inherited intellectual disability and autism spectrum disorder (ASD). CGG expansions of between 55 and 200 repeats, termed premutation (PM: ~1/200 females; ~1/800 males), are associated with an increased risk of having a child with FXS. This risk increases with the size of the PM CGG expansion in females, with some evidence

suggesting that AGG interruptions may modify the chance of expansion.³

PM alleles are associated with fragile X–associated primary ovarian insufficiency (FXPOI), resulting in premature menopause in ~20% of women, and, among individuals over the age of 50, fragile X–associated tremor/ataxia syndrome (FXTAS) in up to 45% of PM males and 17% of PM females.⁴ Depressive and anxiety disorders are also associated with PM alleles.^{5,6}

Additionally, smaller and more common CGG expansions between 45 and 54 CGG repeats, called “gray zone” or “intermediate” alleles (hereafter GZ: ~1/66 females, ~1/112 males) have been proposed to increase the risk of developing both FXTAS-like neurodegenerative disorders and FXPOI.^{7,8}

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However, this is controversial, as evidence of GZ-specific phenotypes is based on a few small studies that were impacted by selection bias.

The primary mechanisms underlying PM (and possibly GZ) presentations are postulated to be distinct from those involved in FXS. In PMs, the most important are aggregation of specific proteins mediated by overexpressed *FMR1* mRNA, mitochondrial dysfunction, overexpression of long noncoding RNA of *ASFMR1/FMR4*, *FMR5*, and *FMR6*, and repeat associated non-ATG translation.^{9–12} However, decreased production of FMRP, previously considered unique to FXS, has also been described in individuals with PM alleles.¹³

Several smaller studies have provided some evidence for a FXS-like phenotype of ASD, developmental delay (DD), attention deficit hyperactivity disorder, and learning difficulties in proband children with PM and GZ alleles.^{14–16} However, these may also be explained by the presence of mosaicism for FM alleles on the background of PM or GZ alleles, which can be missed by standard diagnostic testing protocols, as well as possibly non-*FMR1*-related learning or behavioral disorders.¹⁷ Moreover, while a few prevalence studies have found enrichment of PM and GZ cases in cohorts with ASD and/or special needs,^{18,19} many others failed to replicate these findings, showing no association with neurodevelopmental disorders^{20–23} (as summarized in **Supplementary Table S1** online).

Finally, a number of smaller studies associated alleles of ≤ 26 CGG repeats with an increased risk of developing fertility problems or behavioral problems and/or having children with developmental disability or a psychiatric illness, resembling to a degree the *FMR1*-related phenotypes, FXPOI and FXS.^{24,25} However, these findings are somewhat controversial owing to lack of replication by larger studies.

This study investigated pediatric patients with DD diagnostic test referrals (totaling ~19,000 cases) to determine the frequency of males and females with PM and GZ alleles, with statistical comparison to prevalence data from two population cohorts (newborn screening²⁶ and population carrier screening²⁷). The study hypotheses were that (i) PM and GZ frequencies are significantly enriched in children referred for DD testing as probands and (ii) CGG size distribution is different in a cohort of children referred for DD diagnostic testing compared with the general population.

MATERIALS AND METHODS

Study population

Between the 1980s and the time that this study was conducted, all clinician referrals for DD diagnostic testing at Victorian Clinical Genetics Services (VCGS) included *FMR1* genetic testing as part of the standard protocol. The most common reason for referral was DD, followed by suspected intellectual disability, ASD, language delay, learning disorder, and/or FXS. The first DD cohort was sourced through the Medipath system at VCGS and comprised 10,235 pediatric DD referrals (≤ 18 years old) made between January 2003 and December 2009 (hereafter, DD #1 cohort). These samples are

a proportion of a previously described larger cohort of individuals aged from < 1 week to 89.9 years.²⁸ Because the DD #1 cohort was sourced through an archived database, CGG size was not available. The second DD cohort included 8,841 pediatric DD referrals (≤ 18 years old) to VCGS between September 2013 and April 2017 (hereafter, DD #2 cohort). Data for this unpublished cohort were available through the Laboratory Information Management System (LabWare, Wilmington, DE) and included polymerase chain reaction (PCR)-based CGG sizes.²⁹ Although the protocol of using PCR and Southern blot in FXS diagnostic testing has not changed significantly since 1992 at VCGS, chromosomal testing changed from conventional karyotyping to use of chromosomal microarray testing in 2012. This change to the protocol for DD testing at VCGS prevented pooling of the two DD cohorts. All cases identified as having an FM allele and those with pathogenic chromosome abnormalities identified elsewhere in the genome were part of the exclusion criteria for this study.

The first population cohort comprised 1997 newborns born between November 2009 and December 2010 at the John Hunter Hospital for whom consent for *FMR1* CGG testing was given by a parent or guardian; the consent rate was 94%.²⁶ The second population cohort comprised 14,249 females (17–57 years old) from the general population screened for *FMR1* expansions as part of the VCGS premarital genetic-carrier screen (hereafter called the adult carrier-screening cohort).²⁷ This program offers reproductive carrier screening to women for cystic fibrosis, FXS-associated disorders, and spinal muscular atrophy. Specific details of all four cohorts are provided in **Supplementary Note S1 online**.

Molecular testing protocol

First-line *FMR1* testing at VCGS was performed on blood or saliva DNA. This was conducted using a fully validated PCR amplification assay with precision of ± 1 repeat and limit of detection at 170 CGG repeats in males and 130 CGG repeats in females.²⁹ Second-line confirmatory testing involved Southern blot analysis for amplified repeat sequences in the PM range, and inconclusive PCR results including “one peak” females and “no peak” males.³⁰

All infants for whom samples were included in the newborn-screening cohort were born at John Hunter Hospital in Newcastle, Australia. Extra discs were punched from each child’s newborn-screening sample cards as part of a fragile X feasibility study by the NSW Newborn Screening Programme and Department of Molecular Genetics at the Children’s Hospital, Westmead, Australia. Two PCR methodologies were used to determine CGG size: (i) a modified PCR assay using a chimeric CGG-targeted primer³¹ and (ii) a standard PCR-based fragile X assay³² that was run in parallel to correlate with the chimeric primer assay. Alleles with < 40 repeats were sized by nondenaturing capillary electrophoresis and alleles with ≥ 40 CGG repeats were sized using denaturing capillary electrophoresis.

The adult carrier-screening molecular testing was performed by triplet-primed PCR of the *FMRI* CGG repeat region using the *FMRI* TP-PCR commercial kit (Abbott Molecular, Lake Bluff, IL) or AmpliDeX *FMRI* PCR kit (Asuragen, Austin, TX).³³ Briefly, PCR products were denatured at 95 °C for two minutes after being mixed with a ROX 1000 size standard (Asuragen) and Hi-Di formamide (Thermo Fisher, Waltham, MA). These were then run on ABIPRISM 3730 capillary electrophoresis (Life Technologies, Foster City, CA) using POP-7 polymer (Life Technologies) with a 50-cm capillary, according to the manufacturer’s instructions. Samples that had expanded alleles showed the triplet repeat “stutter” pattern, with CGG sizing determined using Gene Mapper software version 5.0 (Life Technologies). All females identified as being in the PM range were reflexed for confirmatory testing at VCGS using PCR CGG sizing and Southern blot analysis.

Statistical analyses

The equality of the proportions of positive PM and GZ results was computed using Fisher’s exact test. All comparisons that were significant at *p* < 0.05 were then analyzed using pairwise comparisons, also using Fisher’s exact test, where results are presented before and after adjustment for multiple

comparison using the false discovery rate (FDR). Binomial probability test was used to compare the proportions with inclusive and exclusive *FMRI* family history. Because of the small sample size, intergroup comparisons of CGG size in the PM range between the DD #2, adult carrier-screening, and newborn-screening cohorts were performed using a nonparametric Kruskal-Wallis test. All analyses were conducted using Stata version 13 (StataCorp, College Station, TX). PM was defined as CGG size 55–199; GZ was classified as 45–54 CGG repeats.

RESULTS

The two DD cohorts were composed mainly of proband referrals who did not list knowledge of an *FMRI* expansion in a blood relation (Table 1). Each DD cohort also included a proportion for whom no clinical notes were provided (DD #1: *n* = 494; DD #2: *n* = 440). These were included in the main analyses of proband data. In the adult carrier-screening cohort, knowledge of an *FMRI* expansion in a blood relative was indicated on the test request form by a small number of females.

Frequency of males and females with positive *FMRI* PM and GZ results

The PM and GZ frequencies were first determined after exclusion of males and females with a positive *FMRI* family

Table 1 Characteristics of the cohorts

	DD #1 (N = 10,235)	DD #2 (N = 8841)	Adult carrier screening (N = 14,249)	Newborn screening (N = 1997)
Age range (years)	0–17	0–18	17–57	Newborn
% female	23.3%	24.5%	100%	49%
No family history (<i>n</i>) ^a	10,184	8,786	14,228	N/A
Indicated family history (<i>n</i>) ^b	51	55	21	N/A

DD, developmental delay; N, number of individuals in cohort; *n*, sample number; N/A, not available.

^aDD clinical notes and adult carrier screening test request forms do not document knowledge of an *FMRI* expansion in a blood relation. ^bIndicate an *FMRI* expansion in a blood relation.

Table 2 Frequency of PM and GZ results in Australian proband DD and population screening cohorts

	DD #1 <i>n</i> + (<i>n</i> -) % Prevalence	DD #2 <i>n</i> + (<i>n</i> -) % Prevalence	Newborn screening <i>n</i> + (<i>n</i> -) % Prevalence	Adult carrier screening <i>n</i> + (<i>n</i> -) % Prevalence	<i>p</i> ^a
Male					
PM	17 (7,814) 0.22 1/461	8 (6,633) 0.12 1/830	2 (1,014) 0.20 1/507	-	0.331
GZ	53 (7,778) 0.68 1/148	46 (6,595) 0.69 1/144	8 (1,008) 0.79 1/127	-	0.871
Female					
PM	6 (2,347) 0.26 1/392	7 (2,138) 0.33 1/306	8 (973) 0.82 1/123	38 (14,191) 0.27 1/374	0.049
GZ	50 (2,303) 2.13 1/47	34 (2,111) 1.59 1/63	14 (967) 1.43 1/70	357 (13,872) 2.51 1/40	0.009

Data in this table do not include those for individuals in whom presence of an *FMRI* expansion in a blood relation was indicated (refer to **Supplementary Tables S3 and 4** online for *FMRI*-family-history results). DD #1, pediatric DD referrals to VCGS between January 2003 and December 2009; DD #2, pediatric DD referrals to VCGS between September 2013 and April 2017; *n* + (*n*-), number of positive results (number of negative results).

DD, developmental delay.

^a*p* value comparing equality of proportion across the four cohorts was computed using Fisher’s exact test. Significant results *p* < 0.05 were followed up with pairwise analyses (**Supplementary Table S2** online).

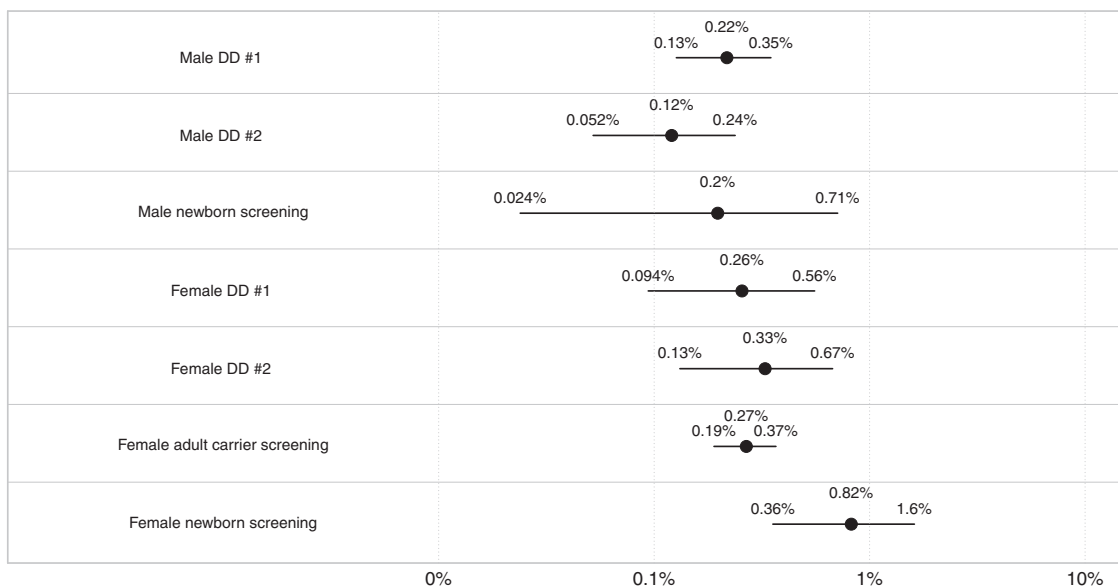


Figure 1 Proportion of PM results with confidence intervals in DD and general population cohorts. Results correspond to **Table 2**. DD #1: pediatric DD referrals to VCGS between January 2003 and December 2009; DD #2: pediatric DD referrals to VCGS between September 2013 and April 2017. Population cohorts: newborn screening; adult carrier screening.

history, indicated either via clinical notes or on the adult carrier-screening test request form (**Table 2**). For males, there was no significant difference in PM and GZ frequency between the DD #1, DD #2, and newborn-screening cohorts (**Table 2** and **Figure 1**).

Pairwise comparison analyses of the female data showed a higher prevalence of PM females in the newborn-screening cohort versus both DD #1 ($p = 0.035$) and adult carrier-screening cohorts ($p = 0.008$) (**Table 2** and **Supplementary Table S2** online). However, after FDR adjustment for multiple comparisons, the difference between the newborn-screening and DD #1 cohorts was no longer significant. There was also a significant increase in the frequency of GZ females in the adult carrier cohort compared with both the newborn-screening ($p = 0.032$) and DD #2 cohorts ($p = 0.008$) (**Table 2**; **Supplementary Table S2** online), with the latter remaining significant after FDR adjustment. Thus in the adult carrier-screening cohort, the number of PM females was depleted, yet that of GZ females was increased.

Importantly, there was no significant increase in the frequency of PM or GZ results in either DD cohort, for males or females (**Table 2** and **Figure 1**). Repeat of all analyses excluding children referred with no clinical notes did not change the results.

Impact of family history of *FMR1* expansion on prevalence estimates

To assess the impact of ascertainment bias on prevalence estimates, the frequency of males and females with PM and GZ results was next determined with inclusion of individuals who had indicated knowledge of an *FMR1* expansion in a blood relation. With this change to the cohorts, male PM and

GZ results remained comparable across the DD and newborn-screening cohorts (**Supplementary Table S3** online).

However, for females, the addition of positive *FMR1* family-history data eliminated the difference in female PM frequency between the DD #1 and newborn-screening cohorts, changing the p value from $p = 0.035$ to $p = 0.324$. It also reduced the difference in PM frequency found between the adult- and newborn-screening cohorts from $p = 0.008$ to $p = 0.017$, with the new p value not significant after FDR adjustment (**Supplementary Tables S3 and S4** online). This analysis shows that the underrepresentation of females with PM alleles in the adult carrier-screening cohort compared with the newborn-screening cohort (reported in **Table 2**) could be artificially related to ascertainment bias.

By contrast, positive *FMR1* family-history data did not influence the differences in female GZ prevalence found between adult- and newborn-screening cohorts ($p = 0.032$). Similarly, the increase of GZ females in the adult carrier screening versus the DD #2 cohort remained statistically significant after FDR adjustment ($p = 0.008$) (**Supplementary Tables S3 and S4** online). Therefore it is unlikely that the greater prevalence of GZ females in the adult carrier-screening cohort (shown in **Table 2**) is related to an impact of ascertainment bias.

Finally, binomial probability tests showed no effect from including the positive *FMR1* family-history data in the analyses, on male and GZ prevalence results. However, in the DD #1 cohort female PMs were more common when the family-history data were included (0.5%, or 1 in 199), compared with when they were removed (0.3%; or 1 in 392) ($p = 0.023$), although this was not significant after FDR adjustment (**Supplementary Table S5** online). A comparison of estimated prevalence rates with and without a positive

family history is presented in **Supplementary Table S6** online.

FMR1 PM CGG size distribution

The median PM CGG size in the DD #2 cohort was CGG 57 repeats (males and females). This was not significantly different from the median CGG size in the newborn screening (CGG: 59.5 repeats) and adult carrier screening (CGG: 61 repeats) cohorts. Analyses of males and females separately did not change these results (**Table 3**).

CGG distribution plots and low normal allele prevalence

Male and female CGG-distribution plots were created for cohorts where CGG size was available (i.e., all except DD #1) (**Figure 2**). Because the focus of these analyses is to understand the lower end of the CGG size distribution and

any differences in the “low normal” allele prevalence across the cohorts, the smaller of the two alleles in females was used for the distribution plots in **Figure 2**. The other allele is presented in **Supplementary Figure S1** online. For each plot the modal value was 29 or 30. In both the DD #2 and adult carrier-screening cohorts, minor peaks at CGG 20 and 23 repeats were observed. These peaks were smaller in males than in females, with the minor peak at CGG 20 reaching ~5% in the male DD #2 cohort, versus ~10% in the female DD #2 and 15% in the adult carrier-screening cohorts.

An analysis of the proportion of individuals with at least one “low normal” CGG repeat size (i.e., ≤26 repeats) was performed (**Supplementary Tables S7 and S8** online). For males, the “low normal” CGG size was found in 19.0% of the DD #2 cohort and 21.3% of the newborn-screening cohort, but this difference was not significantly different ($p = 0.09$).

Table 3 CGG size in PM males and females

	<i>n</i>	DD #2 Median	IQR	<i>n</i>	Newborn screening Median	IQR	<i>n</i>	Adult carrier screening Median	IQR	<i>p</i>
CGG repeat (M and F)	21	57	11	10	59.5	6	44 ^a	61	12	0.3419
CGG repeat (M)	10	57.5	23	2	59.5	9	-	-	-	0.5192
CGG repeat (F)	11	57	11	8	59.5	5.5	44	61	12	0.3658

There were two PMs with a mosaic result for CGG size within the PM range (CGG: 73–85; CGG: 76,157). The analyses above used the mean value for each PM mosaic. Repeat of the analyses using the lowest and highest alleles did not change the results (male and females combined: $p = 0.3419$; females: $p = 0.3658$ to 0.3744); males: $p = 0.5192$.

DD, developmental delay; F, females; IQR, interquartile range; M, males; PM, premutation.

^aAlthough there were no males in the adult carrier screening cohort, these data are presented twice to allow comparisons across the cohorts that include females. Data in this table do not exclude samples with indicated knowledge of an *FMR1* expansion in a blood relation.

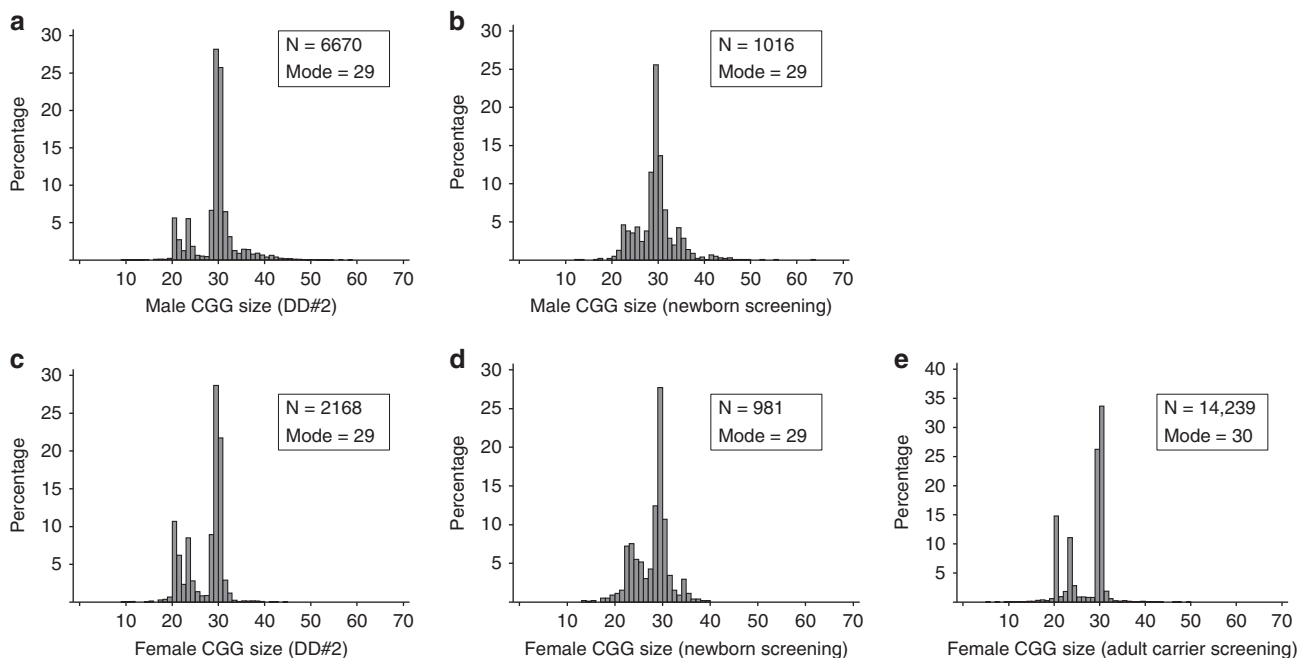


Figure 2 Distribution plots with CGG size on the X-axis (0–70 repeats) and percentage on the Y-axis. Figures show male data from (a) DD #2 ($N = 6670$) and (b) newborn screening ($n = 1016$). Panels c–e show female data (smaller of two alleles): (c): DD #2 ($N = 2168$); (d): newborn screening ($N = 981$); (e): adult carrier screening ($N = 14,239$). Sample size is slightly different from what is reported in **Table 1**, as these data do not include results with > 70 CGG repeats. The larger female allele is shown in **Supplementary Figure S1** online. DD, developmental delay.

In females, similar proportions were found in the three cohorts ($p = 0.61$): 35.0% of the DD #2, 33.6% of the newborn-screening, and 35.0% of the adult carrier-screening cohort. Thus there were very similar proportions across the cohorts of males and females with at least one X chromosome with the “low normal” CGG size allele. Proportions for females with two copies of a “low normal” allele (i.e., both alleles have ≤ 26 repeats) were: 3.0% (DD #2); 5.4% (newborn screening); and 4.1% (adult carrier screening). After FDR adjustment, significant differences remained between the DD #2 prevalence and both the newborn-screening ($p = 0.002$) and adult carrier-screening ($p = 0.011$) cohorts (Supplementary Table S8 online).

DISCUSSION

The present investigation did not find an enrichment of children with PM and GZ alleles in pediatric DD diagnostic referrals to VCGS, compared with two population cohorts.^{26,27} Based on the main analyses, which excluded individuals indicating knowledge of an *FMRI* expansion in a blood relation, the prevalence of PM males in the DD cohorts in Australia was estimated at between 1/461 and 1/830. This is similar to prevalence estimates from the newborn-screening cohort (1/507 males) and those from other groups that have analyzed Caucasian populations in other countries.^{34,35} The proportion of PM females is also similar in DD and population cohorts (~1/306–392), although slightly reduced when compared with prevalence estimates in US populations.^{34,36} There was also no significant difference in GZ frequency in DD versus population cohorts for males and females, excluding an increase of GZ females in the adult carrier-screening cohort versus the DD #2 cohort.

Given the lack of enrichment of PM and GZ males and females in the DD cohorts, there is unlikely to be a clinical phenotype associated with these alleles in children < 18 years old that is significant enough to warrant clinician referral for further DD diagnostic testing. It is therefore unlikely that PM and GZ alleles are a cause of the conditions that are common reasons for clinician referral, such as DD, but also intellectual disability, ASD, language delay, and learning disorder.

The present findings support the results from smaller prevalence studies that also did not find any elevation in PM or GZ frequencies in cohorts with indicated DD and/or ASD.^{20–23} Together, this information challenges the findings from other smaller cohort prevalence studies that have reported a significant excess of developmental problems in children with PM or GZ results.^{18,19} In light of this, generalizability of results from a questionnaire-based study that found an excess of developmental problems in children with PM and GZ alleles but did not perform confirmatory genetic testing is also questionable.³⁷ Furthermore, a hospital linkage analyses study³⁸ and case studies that reported FXS-like features in male PM and GZ probands may have been impacted by selection bias.^{14–16} Differences between studies that have investigated the association between the PM and GZ alleles and developmental problems, which may have

contributed to inconsistency in the literature, include (i) sample size and cohort characteristics; (ii) change over time in awareness of *FMRI*-related disorders by clinicians and availability of diagnostic methodologies; (iii) different definitions of PM/GZ repeat size and different ethnicities; and (iv) use of maternal allele(s) versus an independent typically developing group for control comparisons. Moreover, given that recent studies suggest that mosaicism is more common than previously thought,¹⁷ and difficult to detect using standard testing protocols because of test-sensitivity issues or deletion of PCR primer binding sites associated with somatic mosaicism,^{39,40} it is also plausible that some studies may have unknowingly included individuals who were mosaic for PM and FM, or GZ and FM, which could have skewed the results and interpretation.

This study investigated whether clinical impact in PM children could be driven statistically by a subgroup of individuals who have a very large CGG repeat size that is within the PM range (most individuals have < 70 CGG repeats).³⁴ Given that *FMRI* alleles with > 80 CGG repeats have been associated with increased risk of developing FXTAS and FXPOI,⁶ they may also be related to neurodevelopmental impact in the pediatric setting. However, the median PM CGG size was very comparable among all cohorts analyzed in this study, indicating no link between the larger CGG size and clinician concerns over DD or other common reasons for DD diagnostic testing (e.g., ASD).

The proportion of individuals with the “low normal” allele (≤ 26 CGG repeats) was similar in the DD #2 cohort and both adult carrier and newborn-screening cohorts, consistent with no association between these alleles and neurodevelopmental disorders. Specifically, ~1/3 females and 1/5 males in each cohort had at least one “low normal” allele (~1/ 18–33 females had two copies of these “low normal” alleles). Given the large sample size and replication of proportions across clinical and nonclinical cohorts, the proportions reported in this study are likely to be robust and thus may be useful as a benchmark for future comparisons in investigations of the “low normal” CGG size.

The findings should be interpreted in light of the following limitations. This study cannot comment on the severity of the phenotype in PM and GZ children identified in the DD cohorts, nor can it comment on the involvement of PM and GZ alleles in subtle phenotypes that are unlikely to prompt clinician requests for DD diagnostic testing, such as executive dysfunction, which can impact planning and organizational skills. Indeed, there is emerging evidence that adults with PM alleles are at greater risk of developing subtle visuospatial problems that resemble dorsal stream-processing vulnerability,⁶ plus strong evidence that adult PM females can have elevated, but often sub-threshold, symptoms of social anxiety disorder and major depressive disorder⁵ that may or may not be linked to FXPOI and/or FXTAS.

There is also a possibility of bias in the adult carrier-screening cohort due to (i) higher socioeconomic status, as the carrier-screening test is predominantly offered by private

obstetricians on a test-pay basis, and (ii) PM females who do not have social anxiety or menopausal/infertility problems being more likely to use the adult carrier-screening service.²⁷ Other study limitations include not having the resolution on the nondenaturing capillary electrophoresis to determine whether minor peaks at 20 and 23 were present in the newborn-screening cohort and the possibility that accurate clinical and family-history information was not always provided on the clinician test request form.

In conclusion, there was no statistically significant increase in the frequency of males and females with expanded alleles in the DD clinician referral cohorts compared with newborn and population carrier-screening cohorts. This questions the impact of PM and GZ expansions on FXS-like phenotypes in children, such as DD and ASD. This study also queries the clinical relevance of the recently described “low normal” allele in neurodevelopmental disorders. This is likely to be of interest to families that may have or plan to have children with a PM and GZ allele, such as those identified in population carrier screening or through cascade testing. The present findings also favor not testing for PM and GZ alleles in newborns, owing to lack of sufficient evidence of clinically significant neurodevelopmental impact on these children in the pediatric clinical setting and the potential to identify adult-onset conditions of incomplete penetrance associated with PM alleles.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/gim>

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DISCLOSURE

The authors declare no conflict of interest.

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