Prevalence of Fragile X syndrome among children receiving special education and carrier states in first degree relatives

B Chandrasekara¹, S Wijesundera¹, S S Chong²,³,⁴, H N Perera⁵

(Index words: attention deficit hyperactivity disorder, autism spectrum disorder, CGG repeats, fragile X syndrome, intellectual impairment, special school attendees)

Abstract

Introduction Fragile X syndrome (FXS) is a genetically determined developmental disorder. Underlying genotype is cytosine-guanine-guanine (CGG) repeat expansions with over 200 repeats in the fragile X mental retardation 1 (FMR1) gene. Children with FXS are most accessible in special education institutions in Sri Lanka, with a total of approximately 6000 registered attendees.

Objectives The aim of the current study was to estimate the prevalence of FXS among special school attendees and to screen first degree relatives of affected children.

Methods A nationally representative sample of 850 children (5-18 years) was selected using multi-level stratified sampling. Screening was performed by 3’ direct triplet primed PCR, followed by melting curve analysis. Expanded repeat status of the screened positives were confirmed using capillary electrophoresis, methylation specific PCR and Southern hybridization. Screening of available first degree relatives (n=12) were carried out using the same method of screening and diagnosis.

Results Eleven had FXS. Prevalence of FXS was 1.3% (95% CI 0.9-1.6). Among the 11 with FXS 9 had more than 350 CGG repeats, while the rest had around 300. Twelve first degree relatives consisting of nine mothers, two female siblings and a male sibling were tested. All mothers and female siblings had either full mutation or premutation while the male sibling had CGG repeats in the normal range.

Conclusions Among the special school attendees, prevalence of FXS was 1.3% which has a high risk for autism and attention deficit hyperactivity disorder.

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Identification of carriers in first degree relatives is important in view of the clinical implications of carrier status.

Introduction

Fragile X syndrome (FXS) is a genetically determined developmental disorder and is considered the commonest inherited cause of intellectual impairment. The underlying genotype is cytosine-guanine-guanine (CGG) repeat expansions in the 5’ untranslated region of the fragile X mental retardation 1 (FMR1) gene [1]. There are four FMR1 allelic forms, namely, normal (NL) with 5 to 44 repeats, gray zone (GZ) with 45 to 54 repeats, pre-mutation (PM) with 55 to 200 repeats, and full mutation (FM) with > 200 repeats. Children with > 200 repeats have developmental delay, learning difficulties, seizure disorders and behavior problems. About one-third have autism spectrum disorder (ASD) [2]. Characteristic features of FXS are a long, narrow face, a prominent jaw and large ears. FXS is commoner in males who are more severely affected than females. Males have an intelligent quotient (IQ) of 20-70 [3]. Individuals who are mosaic for FXS may have an above average IQ [4].

Prevalence rates for FXS show wide variation. In the general population, rates vary from 1 in 4000 to 1 in 5000 for males and 1 in 2500 to 1 in 8000 for females [5, 6]. In contrast FXS-PM, prevalence rates are high with 1:130 to 1:256 reported for females, and 1:250 to 1:813 for males [7, 8]. The rates are mostly from West European populations, but figures for other ethnically diverse populations are also known [9-12]. Prevalence rates of South Asian populations are limited. Except for a reported prevalence of 1.05% among an outpatient population of

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children with learning difficulties, the community prevalence for Sri Lanka is not known [13].

Carrier forms of FXS are well known among relatives of affected individuals and are based on recognition of different allelic forms of FMR1 gene and their CGG repeat expansions [14]. A PM allele may expand to FM during maternal transmission, resulting in the birth of an affected child. The risk of expansion to FM show positive correlation with the number of CGG repeats on the PM allele [15]. Accordingly, FM occurs in 13.4%, 20.6%, 57.8%, 72.9% and 97.3% of offspring from mothers with 56-59, 60-69, 70-79, 80-89 and > 90 repeats respectively [14].

Prevalence of PM vary widely [8,16]. PM carriers do not display specific phenotypic features, as in FM. However, approximately 8% of females and 40% of males with PM are at risk of developing tremor/ataxia syndrome (FTAS) in adulthood, which is a neurodegenerative disorder [17]. Also, 20% of females with PM develop premature ovarian failure (POF) [18]. Other known medical and psychiatric features include neuropathy, migraine, sleep apnea, hypertension, hypothyroidism, fibromyalgia, anxiety, depression, obsessive compulsive behavior, hypothyroidism and cardiac arrhythmias [18,19]. In childhood, 10-20% PM carriers have attention deficit hyperactivity disorder (ADHD), learning problems, shyness, anxiety, or autism [20]. Hence, screening for PM in close relatives of FXS individuals is important.

The aims of the current study were to estimate the prevalence of FXS and its behavioural correlates among children attending special education institutions in Sri Lanka and to screen first degree relatives of children with CGG expanded repeats for GZ, PM and FM alleles.

Methods

Selection of sample

A nationally representative sample was identified using multi-level stratified sampling in children attending state and privately sponsored special education institutions distributed in all 25 administrative districts in Sri Lanka. A list of such institutions was obtained from the Statistics Branch of Ministry of Education. The 25 districts were categorized into 3 clusters according to the Poverty Index for districts, which were based on economic and social statistics of the Central Bank of Sri Lanka – 2012. The reason for clustering was to avoid sampling bias due to non-attendance of children from socio-economically deprived areas. Minimum sample size required to determining the prevalence was estimated by analyzing previous reports and also by using the formula \( n = \frac{Z_{\alpha/2}^2P(1-P)}{d^2} \) where, \( Z \) is for normal distribution at 5% \( \alpha \), where P is estimated prevalence of 10% and D is type II error. The calculated sample size was 774, which was equally divided into 3 clusters. The districts and subsequently, the special education units were randomly allocated into each cluster.

Children aged 5 to 18 years were included in the sample. Those with diagnoses incompatible with FXS such as Down syndrome, other genetic disorders, and infective or traumatic insult to brain, were excluded. Information on diagnoses as well as comorbid medical, neurological and behavioral problems was obtained from school records.

Genetic testing for FXS in study sample

All subjects included in the sample were initially screened for FXS using DNA extracted from buccal cells, by performing 3’ direct triplet-primed PCR (3’dTP-PCR) and melting curve analysis (MCA). The individuals who showed positive results were further screened using DNA extracted from venous blood, through capillary electrophoresis (CE), methylation specific PCR (MS-PCR) and Southern hybridization. The screening process involved identification of CGG repeats in the FMR1 gene. Several commercially available test kits and DNA samples were used for this purpose.

Written informed consent for assessment was obtained from parent or guardian of each child. Ethical approval was granted by Ethics Review Committee, Faculty of Medicine, University of Colombo (EC-12-138).

For 3’dTP-PCR and MCA processes, Fast Fra XTM FMR1 identification kit (Biofactory Pte. Ltd, Singapore) was used. Further, to differentiate NL alleles from expanded alleles during MCA, a DNA sample with 43 CGG repeats in the FMR1 gene (Coriell Institute for Medical Research, USA) was used. CE was performed according to manufacturer instructions (Fast FraXTM FMR1 sizing kit, Biofactory Pte. Ltd, Singapore) and thermocycling conditions were applied as described elsewhere [21]. The method described by Zhou (2006) was used to set up MS-PCR conditions [22]. For bisulfite conversion, which is a necessary preliminary step to MS-PCR, manufacturers instructions of EZ DNA Methylation GoldTMKit- Invitrogen USA was followed. Manufacturer instructions (Roche Diagnostics) and method introduced by Sofocleous (2008) was followed for Southern hybridization [23].

Assessment of carrier status of first degree relatives

First degree relatives (biological parents and siblings) of children who were identified with expanded CGG repeats were approached for consent to screen for carrier status. Nineteen relatives agreed for testing and written informed consent was obtained. DNA was extracted from venous blood, using simple salting-out method [24]. Samples were tested for expanded repeats using CE and MS-PCR as described above.

Statistical analysis of data was carried out using SPSS version 17 for estimation of frequency distribution, and calculation of risk ratios.
Paper

Results

Prevalence of FXS among attendees of special-education institutions

The study sample comprised of 850 children. The study flow diagram of selection is given in the supplementary Figure. Mean age of the sample was 10.4 years (range 5-18 years, SD=3.6). The majority, 540 (63.5%), were male.

The prevalence of FXS in the study population was 1.3% (95% CI 0.9-1.6). All positive cases for FXS were males (n=11). Of them, nine children had >350 CGG repeats, while the rest had around 300 CGG repeats. The prevalence among males was 2.0% (95% CI 1.5-2.5). PM alleles (55-200 CGG repeats) were identified in seven children and a GZ allele (45-54 CGG repeats) in one child. All eight children were male. Nineteen children had all subtypes of FXS (including PM and GZ) which gives a prevalence rate of 2.2% (95% CI 2.1-2.3).

With regard to comorbidity in the study group, 135 (15.9%, 95% CI 15.8-15.9) had a diagnosis of autism, 112 (13.2%, 95% CI 13.1-13.3) had attention deficit hyperactivity disorder (ADHD) and 603 (70.9%, 95% CI 70.8-70.95) had physical and behavioural disorders. Among the 11 children who were positive for FM of FXS, autism was an additional diagnosis in 4 (36.4%, 95% CI 35.8-36.9), ADHD in 3 (27.3%, 95% CI 26.7-27.8) and physical and behavioural disorders in 4 (36.4%, 95% CI 35.8-36.9). Risk ratio (RR) was 2.3 (95% CI 2.2-3.2) for autism and 2.9 (95% CI 1.9-3.8) for ADHD. If all subtypes of FXS are considered (including PM and GZ), autism was present in 7 children (36.8%, 95% CI 35.2-38.3) and ADHD in 5 (26.3%, 95% CI 25.8-27.8). Although mental retardation and learning disability was diagnosed in all children, classification regarding the extent as mild, moderate or severe was not available from school records.

Carrier status of first degree relatives

Of the 19 children identified with expanded repeats, 12 first degree relatives of 9 children gave consent for screening. The 12 relatives comprised of 9 mothers (age 30 to 44 years), two female siblings (age 8 and 12 years) and a male sibling (age 5 years). Mutation status and CGG repeats of the relatives and the corresponding children are given in Table 1. None of the screened relatives reported any health problems or being on any treatment.

Discussion

In this nationally representative sample of children attending special education institutions, the main finding was a prevalence rate of 1.3% for FXS FM. Prevalence among males was 2.03%. Prevalence rates ranging from 0.3% to 11.7% have been reported from similar populations of intellectually impaired individuals [10,25]. This wide variation could be explained on the basis of other biological differences in the nature of the study samples as well as the techniques of DNA analysis. For example, high prevalence rate was reported when cytogenetic analysis is the main technique used [10, 26]. Whereas reported prevalence is low when PCR is used [23]. However, prevalence rates vary with any given method. Nevertheless, PCR coupled with Southern blot is generally considered the gold standard in the identification of CGG repeat expansion [27]. In addition high prevalence is seen in severely intellectually impaired populations [23].

Table 1. Profile of expanded CGG repeat elicted from capillary electrophoresis and methylation specific PCR for children with expanded repeats and their first degree relatives

<table>
<thead>
<tr>
<th>Child and the tested First degree relative</th>
<th>Capillary Electrophoresis and Methylation specific PCR findings (CGG repeats/ mutation status)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child 1</td>
<td>(47) GZ</td>
</tr>
<tr>
<td>Mother</td>
<td>(45) GZ</td>
</tr>
<tr>
<td>Child 2</td>
<td>(62) PM</td>
</tr>
<tr>
<td>Mother</td>
<td>(60) PM</td>
</tr>
<tr>
<td>Child 3</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Mother</td>
<td>(109) PM</td>
</tr>
<tr>
<td>Child 4</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Mother</td>
<td>(150) PM</td>
</tr>
<tr>
<td>Child 5</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Mother</td>
<td>(178 ) PM</td>
</tr>
<tr>
<td>Child 6</td>
<td>(300) FM</td>
</tr>
<tr>
<td>Mother</td>
<td>(125) PM</td>
</tr>
<tr>
<td>Child 7</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Mother</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Sister</td>
<td>(300) FM</td>
</tr>
<tr>
<td>Child 8</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Mother</td>
<td>(200) FM</td>
</tr>
<tr>
<td>Child 9</td>
<td>Mosaic for PM (190) &amp; FM (&gt;350)</td>
</tr>
<tr>
<td>Mother</td>
<td>(86) PM</td>
</tr>
<tr>
<td>Sister</td>
<td>(&gt;350) FM</td>
</tr>
<tr>
<td>Brother</td>
<td>(44) NL</td>
</tr>
</tbody>
</table>

GZ-grey zone, PM- pre-mutation, FM-full mutation

The other relevant finding of the study was that the prevalence of autism and ADHD among the FXS positive children was more than twice that of non-FXS individuals. Autism is known to be closely associated with FXS [28]. ADHD in children with FXS also has been studied in individuals with FXS [29]. The findings suggest that the risk of a child with special educational needs having autism is 2.3 times higher and ADHD features is 2.9 times higher if the child also has FXS. Both the findings are of relevance to management of these children clinically and in the school setting.

FXS prevalence was analysed according to the geographical area to estimate whether there are variation between administrative districts. Pockets of higher pre-
valence were evident. In one administrative district (Gampaha), the prevalence was twice that of the geographical area with the lowest prevalence. The geographical distribution was considered important as FXS is the commonest known inherited cause of cognitive impairment in children, but the available data from this study is not sufficient to explain this difference. However, this may indicate the need for further study for carrier states among close relatives. Serious neurological and endocrine disorders later in life are known to be associated with carrier states of FXS.

Screening of available first degree relatives in children with expanded repeats identified asymptomatic carrier mothers and siblings. Mothers of sons with the FM (child 3 to 9) were either FM or PM; majority were PM. Mothers of sons either with PM or GZ (child 1 and 2) were PM and GZ respectively and expansion from mother to offspring has occurred with a small increment of CGG repeats. Mothers of all the nine children were carriers of FXS. The two female siblings had FM and the male sibling had CGG repeats in normal range. There are several possible reasons for these findings. Firstly repeat expansion occurs only during maternal transmission and mother of an affected son is a carrier of the expanded repeats [30]. Secondly degree of expansion depends on the maternal CGG repeat number. The chance is increased with increasing repeat number [15]. Thirdly presence of NL FMR1 gene copy in active X chromosome in addition to the faulty FMR1 gene in inactive X chromosome results the asymptomatic or mildly affected FM females compare to affected FM males [31].

By replicating a previous study we found that small PM (<90 CGG repeats) were expanded to large PM and large PM (>125) were expanded to FM [32, 33]. Although alleles containing CGG repeats less than 54 (NL and GZ) are considered as stable, expansion of 45 repeat to 47 was observed in our study [15, 34]. Similar results where expansion of GZ allele was reported by Fernandez-Carvajal [35]. In his study he found a 52 repeat was expanded to 56.

The study sample was drawn from those attending special education units, which led to exclusion of many who are not in school. Also, exclusion of more prevalent conditions for intellectual impairment, such as Down syndrome in the sampling process could be seen as causing a biased over-estimation in the overall prevalence figure. However, similar exclusion criteria have been used in other studies on comparable populations [11, 12]. The benefit here is that it allows for the apperception of FXS as a possible cause in those with intellectual impairment of unknown aetiology. It is well known that the aetiology is unclear in the majority of children with intellectual impairment. Further, it is not possible to make a diagnosis of FXS clinically alone as shown in a previous study in children with learning difficulties and behaviour problems [13].

**Conclusions**

Among the special school attendees of 5-18 years, prevalence of FXS was 1.3% with a high risk for autism and ADHD. The risk indicates the need for screening for FXS in children with intellectual impairment with associated autism and ADHD. Further the identification of carriers in first degree relatives is important in view of the clinical implications of carrier status.

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**Conflicts of interest**

There are no conflicts of interest.

**References**


