Fragile X syndrome in children with learning difficulties and the diagnostic dilemma

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Abstract

Introduction: Fragile X syndrome (FXS) is the commonest inherited cause of intellectual disability. Children with FXS usually present clinically with developmental, learning and behavioural disorders. Physical characteristics of FXS are well documented and are considered a primary guide to recognition. Genetic screening for FXS targets the detection of cytosine-guanine-guanine (CGG) triplet repeats in the FMR1 gene on the X chromosome.

Objective: Aim was to estimate the frequency, clinically and genetically, in children referred to a specialist child mental health clinic for preschool and school based learning difficulties.

Design and method: A total population of children referred to a specialist mental health service for learning difficulties during a specified period was screened clinically and genetically for FXS. Clinical diagnosis was based on known physical characteristics. They were further assessed on cognitive functions, learning and behaviour. Optimised and validated conventional polymerase chain reaction (PCR) amplification was used for genetic screening where deoxyribonucleic acid (DNA) for the assay was obtained from buccal cells.

Results: The total sample studied was 286 children, 4-12 years of age. Based on morphological features, 5.9% received the diagnosis of FXS and one child was genetically positive. Of the rest, 2 more children were genetically positive but clinically negative. Overall frequency of FXS in the study sample was 1.05%. Similar proportions of children earned additional diagnoses of autism and attention deficit and hyperactivity disorder (ADHD) in the two groups but differed in their cognitive functions.

Conclusions: The overall frequency of FXS in the study sample was 1.05%.

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(Key words: Fragile X syndrome, children with learning difficulties)

Introduction

Fragile X syndrome (FXS) is an X-linked dominant disorder and is considered the commonest inherited cause of intellectual disability[1]. Children with FXS usually present clinically with developmental delay, learning disability and behaviour problems[1,2]. Physical characteristics of FXS are well documented. These include a long and narrow face, large bat-like ears, a prominent jaw and forehead, unusually flexible fingers, flat feet and enlarged testicles in males (macroorchidism) after puberty[3]. Behavioural features described are attention deficit and hyperactivity disorder (ADHD), social interactional and social communication deficits, anxiety and mood changes[4,5]. The intellectual levels in FXS fall into a wide spectrum, ranging from severe disability to mild or borderline[1,2]. Mosaicism has also being identified where intelligence may be within the normal range[6]. Males are said to be more severely affected[1,2].

Genetic screening for FXS is indicated in children with developmental delay, behaviour problems, social communication deficits and learning disability, especially if there is a family history of similar conditions. In most industrialized countries, genetic screening for FXS is routinely done in children with developmental delay, as this knowledge is relevant to providing early intervention and education services. In Sri Lanka, this is not possible due to the low availability of laboratory resources and the high cost involved. Genetic screening targets the detection of cytosine-guanine-guanine (CGG) triplet repeats in the FMR1

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gene on the X chromosome. In almost all individuals with FXS, the disorder is caused by a mutation resulting in an expansion of a deoxyribonucleic acid (DNA) segment within FMR1 as CGG repeats[7]. The number of CGG repeats in normal individuals range from 5 to 50. In FXS full mutation (FM) the CGG segment is repeated more than 200 times. This abnormal expansion prevents the gene from producing FMR1 protein, which is essential for the normal development of the brain[7]. Males and females with 55 to 200 repeats of the CGG segment are said to have FMR1 gene permutation (PM)[7]. The prevalence of FXS on genetic screening of the newborn is estimated as1/3600 to 4000[8].

Prevalence rate of FXS for Sri Lanka has not been studied, despite there being a high prevalence in preschool and school-age children for developmental delay, learning disability, disruptive behaviour disorders and autism[9-11]. The comorbidity for autism in FXS is 33%[12]. Further, identifying FXS children will open an opportunity to screen family members, as females with PM are carriers. The prevalence of female carriers in the general population is 34.4 per 10,000, or 1 in 291[13]. In addition, males with PM are at risk of developing tremor ataxia syndrome (a neuro-degenerative disorder) later in life. Similarly, females suffer from premature ovarian failure[13].

Objective

The objective was to estimate the frequency of FXS, clinically and genetically, among children presenting to a specialist child mental health outpatient setting with learning difficulties.

Design and Method

This was a total population study. Consecutive new referrals to a specialist child mental health outpatient service over a period of 9 months, for preschool / school based learning difficulties as the main presenting complaint as reported by parents and teachers, were included in the study. None had a diagnosis of any specific disorder at the time of recruitment, but those who later earned a diagnosis of autism and other developmental disorders were included in the sample. Children whose learning difficulties were due to cerebral palsy, brain injury or insult, visual or hearing impairment or having a diagnosis or clear morphological features suggestive of a specific genetic / chromosomal disorder other than FXS, were excluded.

Clinical assessment: Clinical diagnosis, based on the presence of documented physical characteristics, was made by agreement between 2 consultant psychiatrists and the cognitive profile was assessed by clinical psychologists. Clinical diagnosis of associated behavioural disorders were also diagnosed using clinical criteria and were classified as mild, moderate or severe, based on its impact on the child's functioning at home and school. Intellectual level was assessed using Test of Non-Verbal Intelligence version 3 (TONI 3). Reading and spelling skills were assessed according to standards set for Sri Lankan children by the National Institute of Education[14].

Genetic screening: Samples of DNA for genetic assay were obtained from buccal swabs after taking measures to prevent contamination with food. The initial screening was done using conventional polymerase chain reaction (PCR) amplification, which was optimized and validated in Sri Lanka in a previous study[15]. Clinically diagnosed cases of FXS where conventional PCR did not amplify CGG expansions were further analysed using Triplet-primed PCR (TP-PCR) and melt curve analysis (MCA) to confirm a positive or negative result[16].

Written informed consent was obtained from parent or guardian. Ethical approval was granted by the Ethics Review Committee, Faculty of Medicine, University of Colombo.

Results

The sample comprised 286 children, 4 to 12 years of age (mean 7 years, SD 3.1). The majority, 236 (82.5%) were male. All children followed normal stream preschool or school education. Profile of morphological and genetic assessment results are given in Figure 1.

According to morphological features, 17 (5.9%) were clinically diagnosed with FXS. On genetic screening one child from this group was positive for FXS. In the clinically negative group (n=269), 2 children were genetically positive for FXS. All 3 were male. Overall prevalence of FXS in the study group (n=286) was 1.05%. Comparison of the clinically positive (n=17) and negative (n=269) groups for FXS is given in Table 1. Intelligence quotient (IQ) in children with autism is not available due to difficulty in obtaining a valid measurement.

Cost of genetic assay of each test sample was calculated by taking into account the utilization of chemicals, laboratory equipment and time, which added up to approximately US$4 (Rs. 524.00).

Comparison of behavioural and cognitive variables between the clinically positive and negative groups for FXS **is** shown in Table 1.

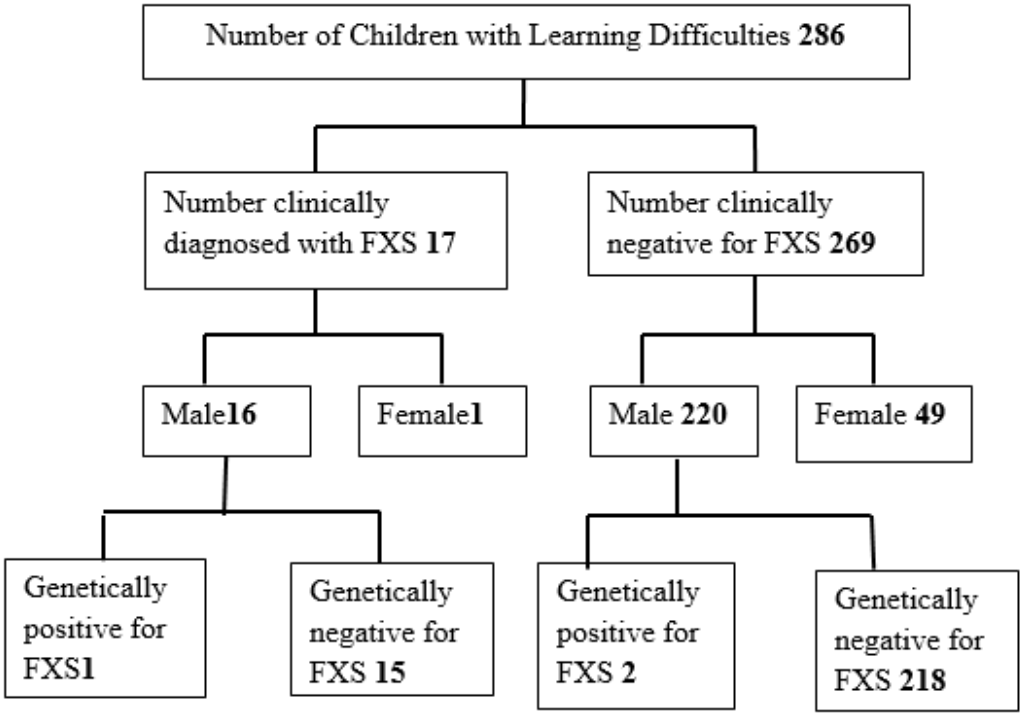

Figure 1: ***Profile of the morphological and genetic results of the study sample***

Table 1: ***Comparison of behavioural and cognitive variables between the clinically positive and negative groups for FXS***

Variable	Clinically Positive (N=17)	Clinically Negative (N=269)
Mean age	6.8 years (SD 2.4)	7.0 years (SD 2.8)
Males	16 (94.0%)	220 (81.8%)
Presence of ADHD	5 (29.4%)	76 (28.3%)
Presence of autism	6 (35.3%)	99 (36.8%)
Presence of reading disability	12 (70.6%)	2 (0.7%)
Presence of multiple problems	5 (29.4%)	57 (21.2%)
Behaviour problems – mild	2 (11.8%)	133 (49.4%)
Moderate	10 (58.8%)	129 (48.0%)
Severe	4 (23.5%)	6 (2.2%)
IQ - >90	0%	93 (34.6%)
70-90	1 (5.9%)	70 (26.0%)
<70	10 (58.8%)	7 (2.6%)
Not available	6 (35.3%)	99 (36.8%)
Genetically positive for FXS	1 (5.9%)	2 (0.7%)

Discussion

The frequency of FXS in the group of children presenting with learning difficulties was 1.05%. A comparative figure is not available as reported prevalence studies are in mentally retarded populations, giving rates of 2.6% to 8.7%[17,18]. A notable finding in this study was the discrepancy between the outcomes of clinical assessment and genetic screening. Although 17 children were identified as fulfilling diagnostic criteria for FXS, only one child was positive on genetic screening. Two other children who had positive genotype for FXS were not identified as such, on application of clinical diagnostic criteria. This poses a dilemma in the diagnosis of FXS, both in terms of over-diagnosis and under-diagnosis, unless all children presenting with learning difficulties to a clinical setting are genetically screened. Comparison of the clinically positive and clinically negative groups (Table 1) shows that both groups are similar in most variables. However, the clinically positive group had more severe behaviour problems and was lower in intelligence. This together with the morphological features would support FXS, but did not correlate with the genetic profile. Problems in correlating clinical and molecular findings in FXS have been described in other studies[19].

At least three possible explanations could be given for the observed discrepancy between clinical and genotypic assessments. Firstly, clinical features described as characteristic for FXS may not be evident in all children having the relevant mutation for FXS. The reason is that the age of appearance of the FXS phenotypic features and their degree of expression vary among individuals[20]. A longitudinal clinical investigation revealed that although genetically positive, the phenotypic features change with age and are most prominent only at 10 to 15 years[20]. In fact, macroorchidism becomes evident only at puberty[1,2]. Accordingly, the only clinical features shared by all 3 genetically positive children were not external characteristics but behaviour problems and learning difficulties, which were common to all 17 clinically positive children as well. Secondly, morphological features similar to FXS are shared by other syndromic and non-syndromic conditions. Sotos syndrome, Prader-Willi syndrome and some causes of autism are given in the differential diagnosis of FXS[12]. Further, typical FXS phenotype without CGG expansions has been identified with FMR1 promoter region deletions and point mutation, which the standard molecular tests are unable to identify[21,22]. Although CGG repeats account for 95% of cases of FXS, the prevalence of point mutations causing FXS is not known[22]. Thirdly, FXS like phenotypes with an associated fragile site on the X chromosome has been described in a different condition called FRAXE syndrome. This syndrome is due to CGG repeat expansions in fragile X mental retardation gene 2 (FMR2), which lies distal to FMR1 and could not be detected by the screening method used in this study. Similar to FXS, a wide range of developmental, learning and communication disabilities have been identified in these children too, with associated attention problems, hyperactivity and feature of autism[23,24].

The genetic screening technique used in this study had noteworthy advantages. Here, buccal cells rather than blood samples were used as the source of DNA. Such a non-invasive method is beneficial in children with learning and behaviour difficulties. Also, assay using conventional PCR provided a low cost method, which also gave rapid results. The comparison is made with "Southern Hybridization" (considered the gold standard for FXS testing), which requires a larger quantity of DNA that cannot be obtained from a buccal swab, and needs 5 to 7 days for the assay[25]. In addition, commercially available "test kits" cost around US$ 18 to 20 (Rs 2358.00 – 2620.00) per assay, whereas the comparable cost in this study was US$4 (Rs 524.00).

Conclusion

The frequency of FXS in the group of children presenting with learning difficulties was 1.05%.

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