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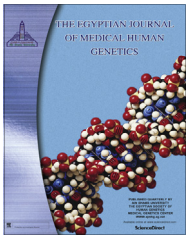

ORIGINAL ARTICLE

Molecular characterization of X chromosome fragility in idiopathic mental retardation

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KEYWORDS

X chromosome fragility;
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Pre-mutation phenotype;
Full mutation phenotype

Abstract *Background:* Fragile X syndrome (FXS) is the most common form of inherited mental retardation. Frequency of fragile X syndrome among male siblings and relatives of mentally retarded patients is relatively high. Cytogenetic diagnosis of FXS is unreliable since it is ineffective for the diagnosis of premutated males or females. Proper molecular diagnosis is a pre-requisite for providing proper counseling advice.

Subjects and methods: Sixty-four males with idiopathic mental retardation, ranging in age from 4.2 to 19 years (10.92 ± 4.00) were clinically pre-selected, based on scoring protocol comprising eight features of the syndrome, before molecular testing. A rapid polymerase chain reaction-based screening was applied for detection of expanded *FMR1* alleles. Samples that did not yield the normal band lengths were subjected to a second PCR screen. The secondary screen utilizes a chimeric primer demonstrating the presence or absence of an expanded allele.

Results: Amplification of FMRI gene by PCR of tested patients revealed that 8 cases (12.5%) have full mutation and 6 cases (9.4%) have premutation. A wide range of Fra X-scoring ranging from 1 to 7 features was detected in examined cases. Significant clinical features included large prominent ears, hyperextensibility of joints and macroorchidism in post pubertal males.

Conclusions: A simplified checklist of fragile X should be used for patients with idiopathic MR and those patients above score 3 should be tested for FXS. The diagnostic assay may be used as a screening method for fragile X syndrome being rapid and cost effective compared to other techniques. In addition, screening of all relatives of proven patients should be performed to detect clinically unidentified cases for provision of proper counseling and optimal management of detected cases.

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1. Introduction

X-chromosome fragility is a genetic defect that underlies the pathogenesis of a wide spectrum of clinical syndromes headed by fragile X mental retardation. The fragile X mental retardation (FMR) gene regulates the synthesis of a protein, fragile X mental retardation protein (FMRP), an RNA-binding protein that plays an important role in regulating proliferation and differentiation of adult neural stem-progenitor cells. The mutation of the FMR gene results in drastic consequences on many neural functions including memory, cognition and behavior, hence the wide clinical spectrum of disorders caused by this genetic defect [1]. The mutational basis of the genetic defect resides in pathological expansion of the normally existing 45–55 repeats of CGG bases in the wild-type FMR gene to many times this number, up to many hundreds, in the genes of patients. The resulting pathophysiological alterations pave the way to the development of the many disorders, notably fragile X syndrome, autism and other less well-characterized neuro-psychiatric phenotypes. Lesser increases, short of 200 repeats, occur in carriers or pre-mutation states [2]. Fragile X syndrome (FXS) is the most common form of inherited mental retardation. Cytogenetic diagnosis of the syndrome depends on visually revealing the fragility of the terminal portion of the long arm of the X chromosome of affected patients in cell culture. However, this cytogenetic diagnostic approach is unreliable, particularly within the context of provision of genetic counseling advice, since it is ineffective for the diagnosis of pre-mutated males or females. On the contrary, molecular diagnosis of the mutational defect of the FMR gene provides an accurate, reliable and definitive diagnosis of the genetic defect [3]. A lot of studies have pointed to the finding of this peculiar genetic defect in many genetically-determined diseases, including autism and psychiatric-behavioral disorders. It has been estimated that fragile X gene mutation, probably, underlies the etiology of autism of 2–6% of autistic children. Even more remarkable is the fact that between 15% and 30% of boys with fragile X syndrome meet the diagnostic criteria of autism [4].

2. Aim of the work

The present study aims at molecular detection of the fragile X mental retardation gene mutations among a sample of Egyptian male patients with isolated idiopathic mental retardation. Inclusion of isolated idiopathic cases of mental retardation in the study is intended for two purposes. First, revealing the incidence of the defect in this category of patients who are subjected to many diagnostic tests, including conventional cytogenetic studies, without being tested for this particular genetic defect, and second, obviating the possibility of skipping their proper molecular diagnosis which is a pre-requisite for providing proper counseling advice to their family members. In addition, the results of the study might contribute to better understanding of genotype–phenotype correlations in male cases with isolated idiopathic mental retardation.

3. Subjects and methods

The present study was carried out on 64 male patients with isolated idiopathic mental retardation selected from outpatient clinic in Neuro motor system National Institute and the Genetics Clinic, Pediatrics Hospital of Ain-Shams University. Their ages ranged from 4.2 to 19 years, with a mean age of (10.92 ± 4.00). Thirty-seven patients (57.8%) were more than 10 years of age. The remaining 27 (42.2%) were less than 10 years of age. Seven cases (50%) were diagnosed at ages 5–10 years.

Selection criteria for isolated and idiopathic mental retardation included: idiopathic mental retardation, absence of pathognomonic features of known recognizable syndromes, absence of signs and/or findings suggestive of chromosomal cause of their disease, absence of symptoms suggestive of underlying metabolic etiology with normal metabolic screen, absence of radio-imaging findings of morphological brain defects, absence of prenatal/postnatal history suggestive of a perinatal insult, including intrauterine infections and absence of postnatal history suggestive of a relevant head trauma or brain infection.

All cases included in the study were subjected to: (1) Complete history taking with special emphasis on aspects related to the idiopathic nature of their mental retardation, including: full details about prenatal, perinatal, immediate post natal history and neonatal intensive care unit admission (NICU), past history including history of head trauma and CNS infections and complete family history, pedigree construction and detailed genealogical study of the family pedigree. (2) Thorough clinical examination was done for all cases including: weight, height, head circumference and examination of the different body systems with special emphasis on neurological examination and eight fragile X (FXS) features. The eight items score used in this study included family history of MR, long face, large prominent ears, hyperextensibility of the finger joints, macroorchidism (in post pubertal males), hyperactivity, autistic features and unusual speech pattern [6–8]. In addition, IQ testing was done according to Stanford–Binet Intelligence Scale test [9]. (3) Mutation analysis of the FMR gene triplet repeat expansion defects was performed according to the technique described by Tassone et al. [5] on blood samples obtained from patients, following informed consent from parents or guardians.

The work has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, and acceptance of The Ethical Committee of Ain-Shams University.

(A) DNA extraction: Whole peripheral blood samples will be collected on EDTA (1 ml on the average) and stored in −20 °C till processing: Genomic DNA was extracted from peripheral blood lymphocytes by spin column method of GeneJET™ purification kit (#K072, PureExtreme ®Fermentas Life Sciences, Thermo Scientific, Vilnius, Lithuania).

(B) Selection and synthesis of oligonucleotides (according to the published nucleotide sequences, and Gene bank data base): The study comprised the application of the screening technique published before [5] for detection of both permutation and mutation statuses of examined cases. Genomic DNA was amplified by PCR with primers c and f using the osmolite betaine according to Fu et al. [10] and Saluto et al. [11].

Primer c (5′-GCTCAGCTCCGTTTCGGTTTCACTTCCGGT-3′).

Primer f (5′-AGCCCCGCACTTCCACCACCAGCTCCTCCA-3′).

Standard primer PCR reactions were performed using the Expand Long Template PCR System (Sigma diagnostics).

Reaction mixtures included hot start Taq polymerase (Sigma diagnostics) 500-mol/L dNTPs, 0.33-M of each primer, and 200 ng of genomic DNA. The PCR buffer also included 2.2 M betaine (B0300; Sigma–Aldrich). The use of modified amino acid betaine is to permit PCR amplification of very GC-rich sequences and due to the stabilizing action on the polymerases, in addition, it allows consistent detection of expanded alleles throughout the premutation range. The PCR products could be directly visualized on agarose [12,13]. It overcomes low levels of contaminants that can co-purify with DNA [14].

In vitro amplification was performed on gradient thermal cycler (HYBAID Express; HYBAID Limited, Ashford, Middlesex, United Kingdom). The PCR cycling profile was as follows: denaturation at 98 °C for 10 min, 10 cycles at 97 °C for 35 s, 64 °C for 35 s, and 68 °C for 4 min; 25 cycles at 97 °C for 35 s, 64 °C for 35 s, 68 °C for 4 min plus a 20-s increment for each cycle; and a final extension at 68 °C for 10 min. The expected PCR fragment region (excluding the CGG repeat region) was 221 bp. The genotypes of the PCR products were determined by electrophoresis on 2% agarose gels stained with ethidium bromide in 1x Tris–EDTA (ethylenediamine tetraacetic acid)-Borate buffer (TBE) against 50 bp ladder molecular weight GeneRuler™ 50 bp DNA ladder (Fermentas™, #SM0373, Thermo Scientific/Fermentas, Vilnius, Lithuania). In case of testing males (like in our study) samples that did not yield the normal band, were subjected to a second PCR screen with the c primer and the C*C*G-chimeric primer (5′-AGCGTC-TACTGTCTCGGCACTTGC(C*C*G)4-3′; targeting the CGG strand of the repeat expansion tract) in place of the *f* primer. The secondary screen demonstrates the presence of an expanded allele. This is also true for mosaic males, both for repeat size (presence of both premutation and full-mutation alleles) and/or for methylation (presence of partially methylated, full-mutation alleles).

The PCR products were all documented by Gel Documentation System and Software for DNA analysis (InGenius Syngene™, UK).

Statistical analysis of data was done using statistical program for social science (SPSS) version 18.0. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used when comparing between two means. Mann Whitney *U* test: for two-group comparison in non-parametric data. Chi-square (χ^2) test of significance was used in order to compare proportion between two qualitative parameters. ANOVA tests were done for comparison among different items in the same group in quantitative data. Probability (*P*-value) *P*-value < 0.05 was considered significant. *P*-value < 0.001 was considered highly significant. *P*-value > 0.05 was considered insignificant.

4. Results

A total of 64 children with idiopathic cause of mental retardation were analyzed in the present study. They presented with a history of developmental delay, cognitive dysfunction, speech and behavioral problems. The age of the patients ranged from 4.2 to 19 years with a mean of 10.92 ± 4.00. There was no significant difference among patients as regards consanguinity (Table 1). Patients' IQ ranged from 20 to 85 with a mean of 63.08 ± 10.61, Table 2. Mild MR was the commonest type of MR 62.5%. There was no significant difference in IQ level between patients with full or permutation (*f* = 0.272, *P*-value 0.763). Out of the 64 mentally retarded patients, 8 (12.5%) children had full mutations, 6 children (9.4%) had pre mutation (Tables 2 and 3).

There was a significant difference in different groups as regards clinical score demonstrating that 37.5% of patients with full mutation had 7 criteria of the score, followed by 25% each for 4 criteria and 6 criteria. In patients with permutation 33.3% had 4 criteria of the score, while in patients with unexpanded allele 26% had 4 criteria and 18% had 6 criteria of the score. There was a significant difference in range of clinical scoring between patients with full mutation and those without expanded allele (Tables 4 and 5).

Comparison of the studied eight fragile X-related features in Fra X males (full and permutation) and non Fra-X MR males showed a significant association between large prominent ears, hyper extensibility of joints and macroorchidism (in post pubertal males), and FXS diagnosis (*P*-value = 0.042, 0.040, 0.003 respectively). Comparison of the studied eight fragile X-related features in Fra X males with full mutation and Fra X males with permutation showed a significant association between large ears, macroorchidism (in post pubertal cases) and long face and FXS full mutation (*P*-value = 0.024, 0.016, 0.031 respectively) with the exception of one boy having permutation with severe autistic features his Cars test was 40 (Table 6). Comparison between the presence of features in those above and below 10 years of age for Fra X positive cases (full and permutation) showed significant difference for macroorchidism and hyperextensibility of joints (*P*-value = 0.010, 0.036 respectively) and insignificant difference for other features. Comparison of the percentage of seizures and/or abnormal EEG finding in Fra X males (full and permutation) and non-Fra-X MR males showed a total of 35.7% of our FXS children had a history of seizures and abnormal EEG with no significant difference (data not shown in tables) (see Fig. 1).

5. Discussion

The target population in our study was patients with idiopathic mental retardation and developmental disabilities, as these characteristics are highly suggestive of fragile X

Table 1 Frequency of consanguinity in the population of the study.

Consanguinity	Unexpanded allele		Full and pre mutation		Total	
	N	%	*N*	%	*N*	%
Negative	32	64	11	78.6	43	67.2
Positive	18	36	3	21.4	21	32.8
Total	50	100.0	14	100.0	64	100.0
Chi-square						
χ^2	1.053					
P-value	0.305*					

* Significant *P*-value < 0.05.

Table 2 FRAXA genotyping among patients.

Results	No. of patients	%
Genotype		
1-Full mutation	8	12.5
2-Premutation	6	9.4
3-Unexpanded allele	50	78.1
Total	64	100.0

Table 3 IQ and MR levels among patients.

Results	No of patients	%
IQ and MR		
1-Subnormal (70–85)	13	20.4
2-Mild (70–50)	40	62.5
3-Moderate (50–35)	10	15.6
4-Profound (below 20)	1	1.6
Total	64	100.0

Table 4 Score for the eight Fra X-related features among patients in relation to degree of mutation.

Scoring	Full mutation		Premutation		Unexpanded allele	
	N	%	*N*	%	*N*	%
1	0	0	1	16.7	3	6.0
2	0	0.0	1	16.7	8	16.0
3	1	12.5	0	0.0	10	20.0
4	2	25.0	2	33.3	13	26.0
5	0	0.0	1	16.7	6	12.0
6	2	25.0	1	16.7	9	18.0
7	3	37.5	0	0.0	1	2.0
Total	8	100.0	6	100.0	50	100.0
Chi-square						
χ^2	19.936					
P-value	0.048*					

* Significant *P*-value < 0.05.

syndrome (FXS). It is of prime importance to screen patients demonstrating symptoms of FXS and to increase the detection rate for this disease. One of the main impediments to the implementation of screening for expanded alleles of the FMR1 gene has been the absence of a rapid, inexpensive screening tool that would be capable of detecting all expanded alleles in both males and females [5].

In the current work, we have applied the new PCR-based approach for FMR1 genotyping that combines modification of the betaine protocol previously described [5,11] with use of CGG-targeted (chimeric) primer. It generates an extensive distribution of PCR products only in the presence of premutation or full-mutation alleles. Providing a single band indicative of a full mutation, has been the goal of previous, but unsuccessful methods. The current method results in a broad smear as a result of amplifications of multiple different lengths. The smear is indicative of the presence of a full mutation. A sample from an individual without an expanded allele will not create a large smear. This method is extremely rapid and permits the test to be integrated, with the routine screening [12]. This means that as a screening tool, all males can be typed with no requirement for a secondary screening tool [5]. Many different restriction enzymes can be used in combination to determine both expansion and methylation statuses for an individual [15]. The main disadvantage of Southern blot - the most accepted method for testing the expanded CGG repeat - is that it requires a large amount of DNA and is laborious, both of which are features that prevent the rapid and inexpensive screening [16]. Compared with Southern blots, the PCR test is inexpensive, automated, and fast. It can be performed on very small amounts of DNA. For many other PCR protocols, the DNA fragment with the expanded repeat does not amplify. This is especially problematic for females and persons with repeat-size mosaicism who could appear to have a single, normal repeat size [17,18]. In the protein-based assay, the percentage of FMRP detected in lymphocytes from blood smears is used to determine affection status [19–21]. Typically, fewer than 40% of the lymphocytes from males with the fragile X syndrome have detectable amounts of FMRP [20]. This protein-based test has been adapted for hair root [22,23] and prenatal samples [24,25]. This technique cannot accurately identify affected females [26]. The currently applied method permits determining whether an individual either has a premutation in the gene known to have 55 to up to 200 repeats and is therefore a carrier, or has a full mutation in the gene known to be above 200 repeats which are generally hypermethylated, and the gene is silenced [27]. A primary screening tool does not need to define the exact size of a full mutation allele, but only to signal its presence. The ability to early diagnose persons with FXS can reduce the burden of this disorder on the individual, on the individual's family, and on society in some embodiments [12].

Table 5 Range of clinical scoring in the population of the study.

	Scoring		ANOVA	
	Range	Mean ± SD	*f*	*P*-value
Full Mutation	3–7	5.50 ± 1.60	3.937	0.025*
Premutation	1–6	3.67 ± 1.86		
Unexpanded allele	1–7	3.84 ± 1.56		
Tukey's test				
Full mutation and premutation	Full mutation and unexpanded allele	Premutation and unexpanded allele		
0.091	0.021*	0.965		

* Significant *P*-value < 0.05.

Table 6 Comparison between the studied eight fragile X-related features in Fra X males with full mutation, permutation and non-fragile X MR Males.

Fragile X-related features	Unexpanded allele (n = 50)		Full and pre mutation (n = 14)		Chi-square		
	N	%	N	%	χ^2	P-value	Sig.
Large ears	24	48.0	11	78.6	4.125	0.042*	Sig.
Hyper extensibility of joints	14	28.0	8	57.1	4.118	0.040*	Sig.
Family history of MR	28	56.0	8.0	57.1	0.006	0.939	N Sig.
Macroorchidism (in postpubertal cases)	3	6.0	5	28.6	5.720	0.003*	Sig.
Hyperactivity	31	62.0	9	64.3	0.024	0.876	N Sig.
Autistic features	18	36.0	8.0	57.1	2.027	0.155	N Sig.
Abnormal speech pattern	42	84.0	10.0	71.4	1.135	0.287	N Sig.
Long face	24	48.0	7.0	50.0	0.018	0.895	N Sig.
Fragile X-related features	Full mutation (n = 8)		Premutation (n = 6)		Chi-square		
	N	%	N	%	χ^2	P-value	Sig.
Large ears	8	100.0	3.0	50.0	5.091	0.024*	Sig.
Hyper extensibility of joints	4	50.0	4	66.7	0.389	0.389	N Sig.
Family history of MR	4	50.0	4.0	66.7	0.389	0.533	N Sig.
Macroorchidism (in postpubertal cases)	5	50.0	0.0	0.0	4.200	0.016*	Sig.
Hyperactivity	6	75.0	3.0	50.0	0.933	0.334	N Sig.
Autistic features	5	62.5	3.0	50.0	0.219	0.640	N Sig.
Abnormal speech pattern	7	87.5	3.0	50.0	2.363	0.124	N Sig.
Long face	6	75.0	1.0	16.7	4.667	0.031*	Sig.

* Significant P-value < 0.05.

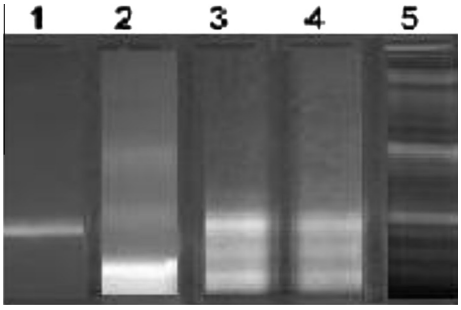

Figure 1 Showing a 2% agarose gel of PCR products. Lane 1: amplified normal PCR product of normal alleles (220 bp) (absent in affected males). Lane 2: a full-mutation, an extensive smear is produced reflecting expanded CGG repeat. Lanes 3 and 4: only small PCR products are produced (expanded alleles). Lane 5: 100 bp DNA ladder molecular weight GeneRuler™.

According to the present study, the prevalence of premutation in general population is estimated at 1 in 813 men [59] and 1 in 259 women [60–62]. Our incidence is much higher probably due to screening of isolated idiopathic MR patients and the selection criteria used. The prevalence of FXS full mutation in examined cases was 12.5% and that of permutation was 9.4%. A cytogenetic analysis, conducted by El Sobky et al. [28] on twenty mentally retarded boys, estimated the frequency to be approximately 20%. A molecular survey reported a frequency of 6.4% of fragile X syndrome among 400 children with mental sub-normality from school age Egyptian males [29]. Another study with reverse transcriptase (RT)-PCR method revealed a frequency of 17% fragile X syndrome full mutation among males with MR [8]. Our results were comparable with a recent study conducted on 53 males with mental sub-normality, speech disorder and score of 16 or higher in Hagerman's checklist [30].The prevalence of fragile X syndrome full mutation was 15% and that of permutation was 5.6% [31].

The frequency of FXS presented in this study is higher than that reported for Caucasian populations where it accounted for 2.6–8.7% among patients with mental retardation [32–34]. Comparing with Far Eastern populations, an inter population diversity was noted in the prevalence of FXS. Our frequency rates were found to be higher than those observed in Chinese mentally retarded patients (2.8–3.2%) [35]. Similar difference was noted between our data and a report of 0.8–2.4% frequency in patients with mental retardation among Japanese population [36,37]. A relatively lower frequency of fragile X syndrome (1.9%) among the mentally retarded individuals was reported from Southern Taiwan [38]. Comparing with Indian population during the last two decades, our frequency rates were found to be higher than that detected by a cytogenetic analysis (2%) [39] and 5.3% frequency of fragile X syndrome among children with unexplained mental retardation [40]. Among Kuwaiti mentally retarded patients the frequency of fragile X syndrome is reported to be 11% [41]. Iqbal et al. [42] reported a frequency of 14.8% in Saudi cases of mental retardation. Among Turkish patients with mental retardation the frequency was 12.8% [43]. Among Iranians, a frequency of 6.3% was reported [44]. The relatively high prevalence of fragile X syndrome within the Arab, other Asian populations and our results may be due to pre selection criteria of study subjects on the basis of clinical phenotype of FXS. Our selection criterion was mental retardation or learning disabilities of unknown etiology. Therefor, the variability of percentage in different studies may be due to difference in sample size, age of the patients, different selection criteria and different detection techniques.

Among our patients, 56.25% of the children had positive family history for mental retardation and 32.8% of mentally retarded children had consanguineous parents, 78.6% of

fragile X patients had non-consanguineous parents despite that consanguineous marriage is high in Egypt as reported by Shawky et al. [45].

In the present study, 57.1% of fragile X children had positive family history for mental retardation either from paternal or maternal side, without parental consanguinity. Different frequencies have been reported elsewhere; 13.9% by Carpenter et al. [46], 3.6% by Froster-Iskenius et al. [47] and 14.8% by Iqbal et al. [42]. Bastaki et al. [41] reported that 85% of patients had affected siblings and 70% of the patients had affected relatives. On the basis of present and past studies, it is apparent that frequency of FXS among the siblings and relatives of mentally retarded patients is relatively high. Pouya et al. [44] observed a familial effect on the prevalence of FXS in children with consanguineous parents, however, offspring of non-consanguineous parents also had FXS. Meguid et al. [29] rated consanguinity to be the major cause for higher rate of FXS among Egyptian subnormal patients.

Assessment of fragile X clinical features revealed that it is not necessary to detect all features in all of the patients at one time. The clinical picture of FXS is fairly characteristic in post-pubertal males. The classical features comprise a triad consisting of long face with large and prominent ears, mental retardation and macroorchidism [48–51]. Clinical diagnosis of FXS in younger children is hindered by the inconsistent expression of the characteristic craniofacial features [51–54]. The presence of different features is reported to vary with age [52,54]. In order to determine whether a set of the most observed fragile X characteristics from the eight-item fragile X checklist would improve selection of individuals at risk, we retrospectively scored all children. Our results showed that the mean score for the fragile X group with full mutation positive findings was 5.5 and 3.67 for fragile X full mutation and pre-mutation respectively. These findings are in agreement with those of Giangreco et al. [6] indicating that genetic analysis of the FXS can be restricted to selected males.

In the present study, the size of the testes was checked. Five fragile X positive cases (35.7%) had macroorchidism compared to 3 (6%) fragile X negative cases. The frequency of macroorchidism in FXS varies from 11% to 80% [41,42,55].

Among studied cases, 57.1% of fragile X children had autistic-like behavior. A relationship between autism and fragile X syndrome has been suggested in various studies [56]. Among autistic patients, 4.3% showed CGG repeat expansion [57]. A comparable incidence (45%) has been reported [41], but most of these patients were in the post-pubertal stage. Other studies reported a lower incidence of autism (10.7%) among fragile X syndrome patients [42]. In addition to mental retardation, which can range from mild to severe, behavioral problems such as hyperactivity, lack of attention, autism spectrum disorders, repetitive and disorderly language have been found to be common in affected males [34,58].

In the present study, the frequency of fragile X-related features among our FXS children (including hyperactivity disorder, perseverant speech/difficulty in speaking words, long face, hyperextensibility of joints and large ears) is consistent with that of Guruju et al. [56] who observed a 15-item checklist of characteristic clinical features for the identification of FXS. Comparable results were detected by Bastaki et al. [41] who found that 85% of fragile X cases had hyperactivity, 85% had defective speech and 90% had large ears. Although seizures were noted as common clinical symptom in our mentally retarded subjects, a total of 35.7% of our FXS children had a history of seizures and/or abnormal EEG finding. These results were in accordance with those of other authors [41].

The clinical presentation of children with permutation was variable. Their IQ ranged between 45 and 80 with variable degree of hyperactivity and moderate to severe anxiety. Similar observation was noted by Yim et al. [63]. It has been documented that children with the premutation alleles have cognitive deficits, behavioral problems and/or autism spectrum disorder [64–66]. Learning problems, developmental delay and/or autistic features have been identified in boys with premutation; these symptoms were observed in our group of boys with premutations. Attention problems in most premutation individuals seem to be milder than those seen in full mutation carriers. This clinical picture may be related to mild fragile X mental retardation protein (FMRP) deficits commonly reported in affected children with the premutation [67,68]. Similar observation has also been reported in some children with diagnosis of autistic spectrum disorder and premutation [64]. Premutation carriers can be detected only by direct molecular analysis of the fragile X mental retardation gene repeat number because they may lack abnormal clinical features as seen in milder forms of FXS such as autistic features and developmental delay [69].

In view of the relatively small number of cases enrolled in this study, more large-scale studies should be carried out to assess the current practice of pediatric screening when there is developmental delay. Larger samples of patients are needed to verify the actual incidence of the disease and the usefulness of the method used in the present study. This assay may be used as a screening method for FXS being rapid and cost effective compared to other techniques A simplified checklist of fragile X should be used for patients with MR and those patients above score 3 should be tested for fragile X. In addition, screening of all relatives of proven patients should be done to detect other clinically unidentified cases is mandatory for provision of proper counseling advice and management protocols for detected cases.

Conflict of interest

The authors declare no conflict of interest. There are no financial or personal relationships with other people or organizations that could inappropriately influence the work.

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