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RESEARCH ARTICLE

Prevalence of fragile X syndrome among patients with mental retardation in the west of Iran

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BACKGROUND: Fragile X syndrome (FXS), an X-linked disorder, is the most common cause of inherited mental retardation. This is caused by a trinucleotide CGG repeat expansion (> 200) on the *fragile X mental retardation 1 gene* (*FMR1*) becoming methylated leading to a deficiency or absence of the FMR1 protein. Determining FXS prevalence in the mentally retarded individuals in the west of Iran was the aim of this study.
METHODS: 200 patients with moderate mental retardation who were clinically suspicious to FXS were screened using cytogenetic and molecular methods. Blood samples were collected and cultured in the specific culture media. The G-Banding method was used for karyotyping and DNA sequencing performed for verifying the results of the cytogenetic tests.
RESULTS: 16 patients (8%) were found to have fragile X syndrome. The results showed that there is no significant association between the fragile X syndrome and economic status and place of residence, however, the relationship between fragile X syndrome and mental retardation in the family history is significant.
CONCLUSION: The frequency of FXS was similar to other reports in the preselected patients. For diagnosis of FXS, chromosome analysis must be accompanied by molecular studies.

Keywords genetic diseases, inborn, *FMR1*, karyotype, diagnosis, mental retardation

Introduction

Fragile X syndrome (FXS) is one of the most prevalent genetic causes of developmental disability, representing the most frequent form of inherited severe cognitive deficit, as a genetic cause of mental retardation after Down syndrome. It is estimated that the FXS affects approximately 1 in 2500 of individuals (Gustavson Kh – Blomquist et al., 1986; Neri et al., 1988; Rousseau et al., 1991). The syndrome is inherited as an X-linked dominant trait with reduced penetrance: 80% of males and 30% of females (Motulsky, 1993). According to studies conducted in Iran, the frequency of fragile X syndrome has been reported as 63% (Pouyaet al., 2009). The syndrome is mainly characterized by a variable degree of mental retardation, typically long and narrow facial appearance with large ears, prominent fontanelles and large testes (Raspa et al., 2017). FXS can be cytogenetically diagnosed by the expansion of X-fragile site at chromosome Xq27.3 (Sutherland, 1977). Indeed, the mutation that results in FXS is the expansion of CGG trinucleotide repeats (> 200) at the 5′ untranslated region of the *FMR1* gene as well as the hypermethylation of the repeats and its flanking region causing the absence of FMR1 protein/FRMP expression (Yu et al., 1991). *FMR1* is a highly conserved gene that is ~38 kb long and consists of 17 exons (Ashley et al., 1993; Eichler et al.). Within the 4.4 kb of the FMR1 transcript, CGG trinucleotide repeats are located in the 5′-untranslated region (5′-UTR). Among normal individuals, these CGG repeats are highly polymorphic in length and constant, often punctuated by AGG interruptions (Fu et al., 1991; Snow et al., 1993; Eichler et al., 1994; Kunst and Warren, 1994; Snow et al., 1994). The normal repeats size differs from 7 to ~60, however, 30 repeats found on the most common alleles. In most affected individuals, CGG repeat is massively expanded over 230 repeats (full mutation) and become abnormally

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hyper-methylated, which results in *FMR1* gene silencing. Alleles with 60 to 230 CGG repeats are called pre-mutation. They are generally un-methylated with normal transcript and protein levels, but extremely unstable during the transitions to the next generations (Feng et al., 1995; Hagerman et al., 2009). Expansion of the pre-mutation to full mutation only occurs in maternal transmissions and depends on the maternal pre-mutation length: because X-linked affected males have more severe phenotypes than affected females, whose phenotype is modulated by activation ratio of normal X chromosome. By identification of other mutations in the *FMR1* gene, such as deletions and point mutations among patients with usual phenotype of FXS but without fragile site expansion, it is firmly established that *FMR1* gene is the only gene involved in the fragile X syndrome pathogenesis (De Boulle et al., 1993; Wohrle et al., 1992). Thus, the absence of *FMR1* gene product i.e. fragile X mental retardation protein (FMRP), is the typical cause of FXS (Hinds et al., 1993). Laboratory diagnosis of fragile X syndrome is done by cytogenetic studies or by molecular methods such as PCR and Southern blot analyses. This study aimed to evaluate the usefulness of the cytogenetic tests prior to molecular tests for the diagnosis of FXS as a trial to reduce the laboratory load of diagnosis. The chance of error detection which can be resolved by the use of molecular methods is 5% (Fu et al., 1991; Lozano et al., 2015; Arvio, 2016) thus, for verifying the results of cytogenetic tests, the samples were tested using DNA sequencing.

Materials and methods

Cases

This study includes 200 mentally retarded males. They were selected from Mental Retardation Center of Iran.

Cytogenetic methods

200 patients with moderate mental retardation who were clinically suspicious to FXS were screened for fragile X syndrome using both of cytogenetic and molecular methods. Blood samples were collected and cultured in the specific culture media. The G-Banding method was used for karyotyping and for verifying the results of the cytogenetic tests, samples were tested by DNA sequencing. The results were analyzed using logistic regression. For routine cytogenetic analysis, 0.3 mL of peripheral blood was incubated in the complete lymphocyte culture medium (10% fetal bovine serum in RPMI 1640, with 0.15% phytohemagglutinin and 1% penicillin/streptomycin) in the 5% CO_2 incubator at 37°C for three days. Metaphases were harvested by adding colcemid for 20 min, followed by hypotonic KCl treatment for 5 min and then fixation using standard 3:1 methanol-acetic fixative (all the reagents were purchased from Sigma-Aldrich). The high-resolution study was performed by synchronization using methotrexate (7-10 M) for 17 h and thymidine (5-10 M) for 5.5 h before harvesting, as previously described (Zhang et al., 1990). For fragile X study, two sets of cultures were examined: a low-folate medium (M 199 culture medium), and a methotrexate-containing medium for the last 24 h of culture (Dewald et al., 1992).

Molecular methods

Genomic DNA from peripheral blood lymphocytes was extracted by the standard method of salting. Primers for amplification of *FMR-1* gene designed and produced (Brown et al., 1993) and sequencing was performed for confirmation. For sequencing, the PCR product for each sample was purified by QIAquick PCR Purification Kit (Qiagen, Cat No: 28104) and then sent to the Pishgam Company for sequencing by pre-designed primers. The sequencing results were confirmed at the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and then analyzed.

Results

In this study, 200 blood samples were taken from the male patients. We confirmed the results of karyotyping by sequencing. The distribution of the mental retardation males according to the time of diagnosis has been shown in Table 1 and the frequency of eight fragile X-related features in the mental retardation males has been shown in Table 2. Nineteen cases (56%) were diagnosed after the age of 10. The karyotyping results showed that 8% (16 cases) suffer from fragile X syndrome. In 200 patients studied, parental consanguinity was found in 8 of the patients (4%), and family history of mental retardation was found in 56 cases (28%); also 172 families (86%) had a poor economic condition. The results of the chi-square test showed that there is a significant association between the fragile X syndrome and the history of mental retardation.

Table 1 Distribution of the mental retardation males according to the age of diagnosis

Age	No. of patients	%
⩽5 y	7	3.5
> 5-10 y	52	26
> 10-15 y	78	39
> 15-20 y	63	31.5
Total	200	100

Discussion

Fragile X syndrome is the most prevalent form of familial mental retardation in the world. Therefore, analysis and recognition of the syndrome carriers have a considerable importance all around the world. Since, there is no statistical

Table 2 Frequency of fragile X features in the mental retardation males

Fragile X-related features	Not seen	Minor	Medium	Severe
Long face	80	31	34	55
Large prominent ears	113	25	18	44
Hypre-extensible joints	75	50	26	63
Macro-orchidism	166	0	0	34
Hyperactivity	21	34	50	95
Autistic features	72	30	37	61
Biting the hand	131	14	15	40
Unusual speech pattern	33	70	31	66

analysis concerning this field in Iran, collecting data on the incidence of the disease has invaluable importance in the disease management, genetic consultation, and designing future plans for the patients. This study determined the frequency of fragile X syndrome among mental retardation subjects in Iran. According to the results of the current study, 8% of the cases had fragile X syndrome. Previous studies on the prevalence of fragile X syndrome, based on cytogenetic diagnosis had reported that, 0.0004-0.0008 percent of men and 0.0002-0.0006 percent of women suffering from fragile X syndrome. According to studies performed in Turkey, 14 of 120 mental retardation cases had fragile X syndrome. However, its frequency in Turkey is 11.7% (Demirhan et al., 2003). Another study performed in Antalya, a province of Turkey, showed that 17 of 132 patients had fragile X syndrome which its frequency is calculated as 12.87% (Bilgen et al., 2005). Studies based on molecular methods determined the frequency of fragile X syndrome in European countries, the US and Australia as 0.6 per thousand (Froster-Iskenius et al., 1983). Jenkins reported the frequency of this syndrome, 6.3% among mental retardation cases and Butler reported the frequency as 6.4% (Jenkins et al., 1994; Butler and Hamill, 1995). In selected populations of mentally retarded patients, an overall prevalence rate of 4.8% of fragile X was reported by Proops et al. (Proops and Webb, 1981). Carpenter et al. studied 36 patients with a family history of mental retardation and found that 13.9% to be fragile X-positive. Iqbal et al. studied 81 patients with a family history of mental retardation; among them, 12 patients (14.8%) were found to be fragile X-positive, which is similar to the Carpenter et al. report (Carpenter Nj – Leichtman et al., 1982; Iqbal et al., 2000). Froster reported that only 3.6% as the frequency in a similar study which was performed on 200 mental retardation cases with a positive family history of the disease (Froster-Iskenius U – Felsch et al., 1983). In this study, 12 of 16 cases of fragile X syndrome patients had family history of mental retardation. In studies which performed in various countries, there were some specific phenotypes defined as patients with fragile X syndrome, which were the primary criteria to diagnose the patients. Our study in Iran showed that most the of men suffering from fragile X syndrome had large testis, large ears, long faces, attention disorders and weak eye contact. Until now, there is no cure for fragile X syndrome, although appropriate decisions and drugs can improve the quality of life in the affected individuals (Grigsby, 2016). The increased understanding of the molecular mechanisms of fragile X syndrome has led to the development of novel therapies. Evidence from mouse models showed that mGluR5 antagonists (blockers) can rescue dendritic spine abnormalities and seizures, as well as cognitive and behavioral problems which may be promising in the treatment of the disease (Dolen et al., 2010; Dolen et al., 2007; Jiraanont et al., 2017). Two new drugs, AFQ-056 (mavoglurant) and dipraglurant, as well as fenobam are currently undergoing human trials for fragile X syndrome treatment (Budimirovic and Kaufmann, 2011; Berry-Kravis et al., 2016). There is also early evidence for the efficacy of arbaclofen, a GABAB agonist, for improving individuals with FXS (Eichler et al., 1993; Jiraanont et al., 2017). Management of FXS may include speech and behavioral therapies, special education, or individualized educational plans, and if necessary, treatment of physical abnormalities. Families with affected fragile X syndrome members are advised to seek genetic counseling to assess the likelihood of having affected children, and how severe any impairments may be in the affected descendants (Hagerman et al., 2009). Since one of the main causes of mental retardation in the world is FXS, thus proper planning and genetic counseling can be a step toward reducing the birth of new cases.

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Conflict of interests

The authors have no conflict of interests. The current study was approved by the ethics committee of Ilam University of Medical Sciences following the principles of the revised Declaration of Helsinki. Written informed consent obtained from each participant for sample collections and data usage for performing this investigation.

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