Prevalence of fragile X syndrome among school-age Egyptian males

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**Background:** Fragile-X syndrome (FXS) is the most common inherited form of intellectual disability. Population-based studies have suggested that the prevalence of the full mutation ranges from 1/3717 to 1/8918 Caucasian males in the general population. The present study is the output of a project aimed at identifying the prevalence rate of fragile-X males in Egypt. A two-step selection with questionnaire and photography was done by trained health visitors and social workers.

**Methods:** A total of 20,500 males were screened. The original work involved inhabitants of the three different large governorates: Cairo (Shobra and Rod El-Farag districts), Giza (Dokki district) and Suez (Suez district). The participants included students of primary and high schools. According to school records, parent reports, and clinical examinations, 130 males were selected and subjected to molecular analysis after informed consent was obtained from care givers.

**Results:** The prevalence of FXS mutation among Egyptian males was 0.9 per 1000. Moreover, it was 6.4% among mentally subnormal males.

**Conclusions:** The high prevalence of FXS necessitates special education for affected children. There is currently no cure for FXS, however, an individualized treatment plan, beginning during preschool years, can help affected children to reach their full potential.


**Key words:** Egypt; fragile X syndrome; males; prevalence

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**Introduction**

Fragile X syndrome is an X-linked dominant disorder with reduced penetrance, is one of the most common forms of inherited mental retardation. The cognitive, behavioral, and physical phenotypes of this syndrome vary by sex, with males being more severely affected because of the X-linked inheritance of the mutation. Population-based studies suggest that the prevalence of the full mutation, the disorder-causing form of the repeat, ranges from 1/3717 to 1/8918 Caucasian males in the general population. The disorder-causing mutation is the amplification of a CGG trinucleotide repeat in the 5′ untranslated region of the fragile X mental retardation protein 1 (FMR1) gene located at Xq27.3. The fragile X CGG repeat has four forms: common (6-40 repeats), intermediate (41-60 repeats), premutation (61-200 repeats), and full mutation (>200-230 repeats). When expanded to over 200 repeats (full mutation), the repeat region and the adjacent promoter CpG island become hypermethylated, rendering FMR1 transcriptionally inactive and silencing of the FMR1 promoter. Although the events that trigger local CGG expansion remain unknown, the stability of trinucleotide repeat tracts is affected by their position relative to an origin of DNA replication in model systems.

The clinical diagnosis of fragile-X syndrome is not straightforward since dysmorphic features are usually subtle, particularly in young children. The features become prominent as the child grows up. However, a complex mixture of physical, cognitive and behavioral features characterizes the phenotype of patients with fragile-X syndrome. Patients with fragile-X syndrome show variable mental disability, large ears and hyperextensible metacarpo-phalangeal joints.

An established protocol in different centers worldwide is practiced to check for FXS in children with mental sub-normality or developmental delay. The number of individuals found to be affected is related to the size of the population of the same age from which they are drawn. An extensive review of the literature shows a large variability in the FXS prevalence of different populations. The present study aimed at identifying the prevalence of FXS among Egyptian males for the first time.
Methods
A total of 20,500 males, representing general male population, were screened to select those having mental deficits, learning disabilities or emotional problems. They represented three governorates; 17,500 subjects from Cairo (Shobra and Rod El-Farag districts); 2200 subjects from Giza (Dokki district) and 800 subjects from Suez (Suez district). The exclusion criteria included other causes of mental retardation like Down syndrome and severe neurological deficits. The inclusion criteria, according to school records, parent reports, and clinical examination, were: developmental delay, cognitive impairment, language disorder, attention deficit, hyperactivity and learning disability.

Examinations
The following examinations were given to suspected males: thorough medical and family history; three generation family pedigree construction; full clinical examination; comprehensive evaluation (neurological and psychometric) of mental, cognitive, and motor abilities with assessment of linguistic and social skills; IQ testing using Stanford Binnet Test; Illinois Test; Hagerman's clinical checklist[4] for all suspected males. This checklist included 13 items (Cases were suspected and selected as FXS when fulfilling more than five of the following checklist's clinical criteria): mental retardation; hyperactivity; short attention span; tactilely defensiveness; hand-flapping; hand-biting; poor eye contact; perseverative speech; hyperextensible metacarpophalangeal joints; large or prominent ears; large testicles; Simian crease or Sydney line; and family history of mental retardation. Proper genetic counseling will be offered for families proved to have cases with fragile X syndrome.

Polymerase chain reaction (PCR) in selected cases depending on Hagerman's checklist
Total genomic DNA was extracted from peripheral blood using the standard salting out method. Briefly, 3.5 ml of blood on EDTA was transferred into a 50 ml falcon tube. Erythrocyte lysis buffer was then added to reach a volume of 45 ml and incubated at 4°C for 20 minutes, followed by spinning at 1200 rpm at 4°C for 10 minutes. WBC pellet was washed twice with PBS and lysed in 3 ml cell lysis buffer, 2 ml 1 mol/L NaCl, 200 μl 10% SDS and 50 μl Proteinase K followed by incubation at 56°C for 2 to 3 hours. One ml 5 mol/L NaCl was added with thorough shaking and spun at 3500 rpm for 30 minutes at 20°C. Supernatant was mixed with 2 volume of ice cold absolute ethanol to precipitate the genomic DNA. The latter was washed twice with 70% ethanol, spun at 3500 rpm at 4°C for 10 minutes and left to dry then dissolved in 300 μl double distilled water. A standard amplification protocol for CGG repeat region within the first exon of human FMR1 gene was performed according to the method described by Chong et al[5] with minor modifications. In summary, the reaction was performed in a 50 μl mixture, containing 200 mM from each of dATP, dCTP, dGTP, dGTP/7-deaza-dGTP was added at a ratio of 150 mM/50 mM. Gene specific primers were added at 10 pmol of forward and reverse primers. Primer sequences were as follows: forward primer, 5'-GGA ACA GCG TTG ATC ACG TGA CGT GGT TTC-3’; reverse primer, 5’-GGG GCC TGC CCT AGA GCC AAG TAC CTT GTT-3’. The reaction was carried out in a 1 × buffer supplied with PFU polymerase (Stratagene, USA), and 10% DMSO. After addition of 2 drops of mineral oil, the mix was heated at 99°C for 10 minutes (Hot start), and 2 units PFU polymerase was added under oil. Amplification program was as following: 30 cycles of 99°C for 1 minute, 63°C for 90 seconds, 75°C for 2 minutes followed by a final extension at 75°C for 5 minutes. The amplicons were resolved on 2% agarose, stained with ethidium bromide and visualized with UV transilluminator. Normal alleles (20-50 CGG repeats) provided a fragment length of 400-490 bp. To assume that genomic DNA used in the study is intact, we utilized SRY primers. This is to check for successful amplification of genomic DNA from male subjects included in the study. Full mutant alleles (>200 CGG repeats) provided fragment of more than 940 bp (always undetectable on gels) (Fig.).

Results
From 20,500 screened males, 400 (age range: 5-14 years) were selected in accordance with the inclusion criteria
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(250 had mental subnormality and 150 had learning disabilities). History inquiry, examination, exclusion criteria and application of Hagerman's checklist to selected cases revealed that 130 males, 37 from Cairo (Shobra and Rod El-Farag districts), 82 from Giza (Dokki district) and 11 from Suez (Suez district), were suspected to have FXS. PCR of the FMR1 gene was done for the suspected 130 males and the frequency of FMR1 mutation was 0.09% (19 males, 19/20 500) (16 cases had mental subnormality and 3 with learning disability). On the other hand, the prevalence rate of FXS among mentally subnormal males was 6.4% (16/250).

Discussion

Many of first ascertained cases of FXS were of Northern European descent. Subsequently affected males have been identified in all populations and ethnic groups studied with different frequencies. An extensive review of the literature shows a large variability in FXS prevalence from population to population according to the presence of a founder mutation from a common ancestor or a de novo mutation. Although generally consistent, variation among different studies may reflect both chance variation and differences in how the studies were carried out. It also depends on the selection of cases for study aided by the chosen inclusion criteria. Many epidemiological studies from various geographical areas all over the world have previously been reported using DNA methods.

The best protocol is to amplify DNA using polymerase chain reaction. DNA analysis improves diagnostic accuracy (being a sensitive and cost effective tool) and genetic counseling in fragile X families. Major progress in molecular diagnosis has been made soon after the availability of FMR-1 gene cloning and a direct molecular test became available which is confirmatory for fragile X diagnosis. However, for many PCR protocols, the DNA fragment with the expanded repeats does not amplify. This is especially problematic for females and persons with repeat size mosaicism who could be misdiagnosed as normal.

In the present study, the prevalence of FXS mutation, dependent on DNA molecular study, among males was 0.9 per 1000 males, which is close to the results reported by many other authors: 1 per 1000 males, 1 per 1000, 1 per 1200, 0.8 per 1000, and 0.83 per 1000. However, our result is higher than that reported by others: 0.37 per 1000, 0.25-1 per 1000, 0.39 per 1000 and 0.41 per 1000.

Recent studies have shown that the prevalence of FXS in developed countries seems to be declining slightly. This may be due to detection of the faulty gene prior to conception. The prevalence of FXS among Egyptians suggests a high mutation rate which, in turn, may be attributed to a founder effect of this disease. This finding could be attributed to a larger number of founder mutations in our population. However, it needs to be verified by further studies. Zhong et al had assumed that fragile X syndrome in Chinese populations, as in the Caucasian population, may be derived from founder chromosomes. Search for similar figures in different countries around the Mediterranean basin would lend further support to this assumption.

In 1993, Smits et al and Buyke et al failed to show any new mutations for 84 and 68 studied probands respectively. The lack of new fragile X mutations implies that there should be many more fragile X gene carriers in the population than had previously been realized. Linkage disequilibrium was found in the Australian and US populations between the fragile X mutation and adjacent polymorphic markers, suggesting a founder effect of the fragile X mutation. Moreover, Buyke et al found significant linkage disequilibrium in unrelated fragile X patients between the fragile X mutation and an adjacent polymorphic marker. They suggested that a founder effect of the fragile X mutation also exists in the Belgian and Dutch populations. A founder effect was also reported in the eastern Finnish population. Both the absence of new mutations and the presence of linkage disequilibrium suggested that a few ancestral mutations are responsible for most of the patients with fragile X syndrome in the aforementioned populations. On the other hand, Tseng et al suggested that a few ancestral mutations are responsible for most of the patients with fragile X syndrome in the aforementioned populations. On the other hand, Tseng et al had suggested that the relatively low prevalence of FMR1 mutant in a population like Taiwan could be due to the lack of founder fragile X syndromes.

Moreover, in our study, the prevalence of FXS within the mentally subnormal males was found to be 6.4%. The present results were compared with other published data (Table). The relatively high prevalence of FXS within Arab mentally subnormal males may be attributed to the decreased awareness of early detection and prediction of genetic disorders together with the increased percentage of consanguineous marriages among the Arabs, which is a common cause of many disorders with mental subnormality.

Our findings, therefore, have led us to stress upon the significance of early developmental screening, guided by clinically-based checklists, to determine whether a child who is experiencing any developmental or behavioral problems has fragile X syndrome. Genetic screening would result in more positive outcomes. For all families, the average age of diagnosis of FXS was 57 months. This figure declines significantly for families of children born after 1990, to 34 months. The
This project has been approved by the Local Institutional Review Board for Human Rights of Cairo. Ethical approval: This project has been approved by the Local Institutional Review Board for Human Rights of Cairo.

Contributors: MNA proposed and designed the study. AER and DAA wrote the first draft of the paper. They provided advice on medical aspects supervised by MNA. Lab experiment was performed by EMK. All authors contributed to the scientific discussion.

Table. Prevalence rates of FXS among mentally subnormal males in different studies

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
<th>Prevalence (%)</th>
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</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>The present study</td>
<td>6.4</td>
</tr>
<tr>
<td>Kuwait</td>
<td>Tayel[24]</td>
<td>3.5-8</td>
</tr>
<tr>
<td>Kuwait</td>
<td>Bastaki et al[25]</td>
<td>11</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>Iqbal et al[26]</td>
<td>8.5</td>
</tr>
<tr>
<td>Asian population</td>
<td>Sherman[7]</td>
<td>0-11</td>
</tr>
<tr>
<td>Hawaii</td>
<td>Proops et al[27]</td>
<td>4.8</td>
</tr>
<tr>
<td>Japan</td>
<td>Hofste et al[28] and Namba et al[29]</td>
<td>0.8-2.7</td>
</tr>
<tr>
<td>China</td>
<td>Zhong et al[25]</td>
<td>2.8</td>
</tr>
<tr>
<td>Turkey</td>
<td>Tuncobiilek et al[30]</td>
<td>3</td>
</tr>
</tbody>
</table>

In conclusion, the criteria needed for proper estimation of prevalence rate of FXS should depend on proper selection of cases using proper clinical checklists and the use of sensitive molecular techniques in detecting gene mutations. Further population-based studies in diverse populations are necessary to explore the possibility that the prevalence of fragile X syndrome differs in different populations around the world. Screening for fragile X syndrome is important to reduce the birth prevalence of the disorder. Early diagnosis of FXS is indicated to overcome the subsequent pregnancies after diagnosis of the first affected child.

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References
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