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THE PREVALENCE OF FMR1 PREMUTATION/FULL MUTATIONS IN JORDANIAN PATIENTS WITH COGNITIVE IMAPAIRMENTS, MENTAL RETARDATION, OR PREMATURE OVARIAN INSUFFICIENCY (POI).

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INTRODUCTION

The fragile X syndrome (FXS) is defined as a loss of function disorder. The expansion and subsequent methylation of a CGG repeat in the 5' untranslated region (5'UTR) of the fragile X mental retardation 1 (*FMR1)* gene (chromosome locus Xq27), results in the deficit of the *FMR1* gene-specific product. In general, the diagnosis of *FMR1*-related disorders, including POI in women, depends on the detection of a dynamic alteration in the *FMR1* gene, including normal range alleles (6-50 CGG repeats), large sized permutations (50-200 CGG repeats), and full mutations (>200 CGG repeats) (***Fig. 1***) (Oostra & Willemsen; 2008). Although several studies have been conducted to investigate the phenotype-genotype relationship in different populations, precise prevalence figures of the *FMR1* premutation and full mutation alleles, and their association with *FMR1*-related disorders in Arab populations, are lacking (Bastaki *et al.,* 2004; Iqbal MA *et al.,* 2000). We report on a series of Jordanian subjects presenting symptoms ranging from learning disabilities to severe mental retardation, and 8 Jordanian POI patients, all of which were examined for X-chromosome abnormalities and the number of CGG repeats within the *FMR1* gene between 2000 and 2010.

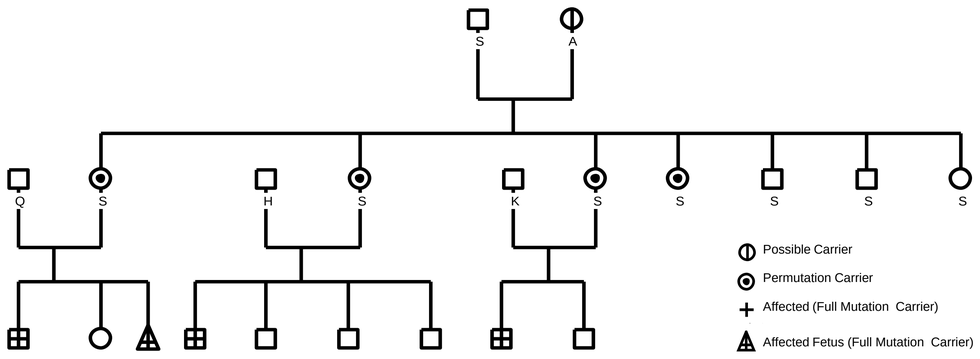

Figure 1, A Jordanian Family Pedigree Highlighting the CGG Triplet Repeat Pattern of Inheritance. The change from normal CGG copy number to full mutation is a multistep process preceding over several generations characteristic of a dynamic mutation.

MATERIALS & METHODS

Participants &Samples

• **392** Jordanian patients presenting symptoms with mental retardation/developmental delay/clinical suspicion of fragile X syndrome were screened between 1989 and 2010.

• **8** Jordanian women that suffer from POI, referred by an endocrinologist for chromosomal abnormalities and *FMR1* gene analysis during 2010.

Cytogenetic:

Cytogenetic analyses of **313** samples was preformed, using excess Thymidine. Samples were considered positive when 4% or more of the cells were found to express the fragile X site at q27.3.

Southern Blot Analysis

60 subjects were examined by southern blot analysis, using the Stb 12.3 probe for hybridization on EcoRI - EagI double digested DNA.

PCR Analysis

50 subjects were examined by PCR, using primers flanking *CGG* expansion in the *FMR1* 5'UTR (***Fig. 2***). The amplification products were run on automated sequencer (Saluto *et al*., 2005)

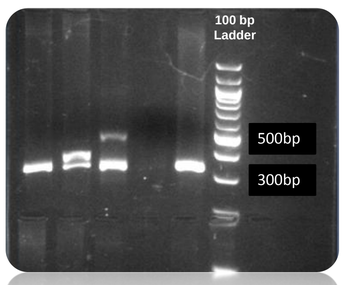

Figure 2, Detection of PCR products from the amplification of the CGG region of the *FMR1* gene. 10 microliters of PCR product was loaded per lane: **1**, Normal male (30 repeats); **2,** Normal female (31/38 repeats); **3,** Premutation female carrier (30/76 repeats); **4**, Full mutation male carrier (>200 repeats); **5,** Full mutation female carrier (31/200> repeats); **6,** 100-bp ladder molecular weight marker.

RESULTS

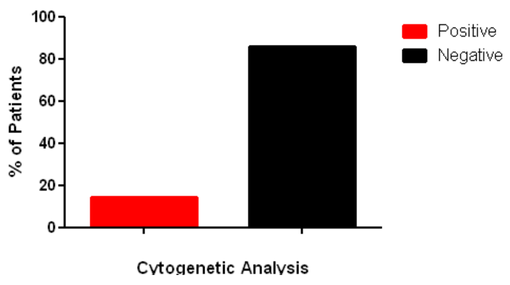

Figure 3, Cytogenetic Analysis of 313 Patients with Mild to Severe Cognitive Impairments. 45 patients (14%), 39 males (12%) and 6 females (2%), were found to express the fragile X site at q27.3.

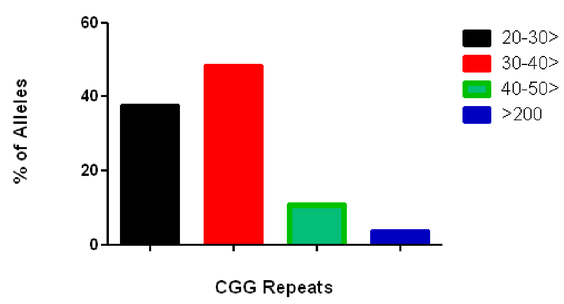

Figure 5, PCR and Sequencing Analysis of 43 Patients with Mild to Severe Cognitive Impairments. 21 (38%) and 27 (48%) subjects had a CGG repeat allele in the range of 20-30 and 30-40 respectively, whereas 6 (11%) subjects had a CGG repeat allele in the range of 40-45. 2 (4%) subjects only had >200 CGG repeats.

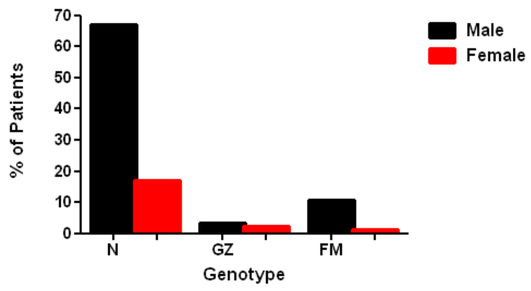

Figure 4, Molecular Genetic Analysis of 97 Patients with Mild to Severe Cognitive Impairments. 65 males and 16 females carried alleles within the normal range, 3 males and 2 females carried alleles in the gray zone (GZ), and 10 males and 1 female carried the fully mutated (FM) allele .

Symptoms	Absence of or Irregular Menses	Abnormal Karyotype	FMR1 Premutations	FSH Level >21.5 MIU/ml	Small or Absent Ovaries	Infertility
Number of Patients	8	0	1	6 (1 not examined)	4 (4 not examined)	3 (5 unmarried)

Table 1, Clinical Analysis of 8 Patients with POI.

DISCUSSION

Among the cognitively impaired subjects, 45 and 11 patients were confirmed fragile-X-positive by cytogenetic and southern blot analysis respectively, and surprisingly no carriers with premutations were observed. Interestingly, 6 of these symptomatic patients are unrelated individuals, with familial origins from the city of Karak, south of the capital Amman. Although further analysis is necessary, preliminary data indicates that Jordanian patients with mental retardation are carriers of the *FMR1* full mutation allele, whereas patients showing mild to moderate cognitive disabilities are within the normal polymorphic range. Among the POI cases examined, the CGG repeat numbers were within the normal range, and only one female carried a premutated allele. Therefore, *FMR1* premutations seem to be uncommon in sporadic cases of POI with no family history of FXS.

CONCLUSION

In general prescreening studies have proven to be very efficient in reducing the prevalence of FXS in populations were the *FMR1* premutation frequency is high. Usually the identification of an individual in which the *FMR1* repeat site is expanded triggers genetic counseling throughout the pedigree and subsequent prenatal testing. Therefore, future studies focusing on the prevalence of premutations within females of normal intelligence in different Arab populations are necessary. In addition, accumulating evidence associating candidate genes and X-chromosome abnormalities with POI necessitates screening of *FMR1* premutations and cytogenetic analysis as part of the routine work-up for any women presenting with POI. Furthermore, comprehensive clinical, histological, morphological, and genetic studies relating to POI patients in Arab populations would be very informative.

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