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RESEARCH NOTE

The AGG interruption pattern within the CGG repeat of the *FMR1* gene among Taiwanese population

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Introduction

The fragile X syndrome is the most common form of inherited mental retardation and is mainly due to an expansion of a (CGG)*n* trinucleotide repeat located in the 5′UTR of the fragile X mental retardation 1 (*FMR1*) gene at Xq27.3 (Kremer *et al*. 1991; Yu *et al*. 1991; Fu *et al*. 1991). In healthy individuals, the (CGG)*n* repeat in *FMR1* is highly polymorphic, with allele sizes ranging from 5 to 50 repeats (Fu *et al*. 1991; Mandel and Biancalana 2004). However, two other stages of fragile X syndrome exist: the premutation stage of 50 to 200 repeats; and a full mutation, which is a massive expansion beyond 200 repeats, that causes mental retardation in males and in about 50% of female carriers. Apart from the length of the repeat, other factors, including AGG interruption pattern, length of uninterrupted CGG, and *DXS548-FRAXAC1* markers, also have impact on the stability of the *FMR1* gene and exert influence on the syndrome (Mandel and Biancalana 2004).

The AGG interruptions in CGG triple repeat occur once in every nine or ten CGG repeats. Most normal alleles have two or more AGG interruptions in five to 50 CGG repeats, while most permutation alleles (50 to 200 repeats) have only a single or no interruption (Eichler *et al*. 1995; Zhong *et al*. 1995). The interruption is associated with certain haplotypes and is related to instability, which can lead to an expansion and eventually, the fragile X syndrome (Eichler *et al*. 1995; Zhong *et al*. 1995; Larsen *et al*. 2000).

Because the total length of the repeat is not a clear predictor of expansion, it is important to determine both the length and the interruption pattern of the repeats to evaluate the tendency of instability. Therefore, this study was conducted to evaluate the pattern of CGG repeats in Taiwan.

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Methods

This study was approved by the IRB committee of Fooyin University. Blood samples were obtained from 78 healthy-male Taiwanese. Genomic DNA was extracted from whole blood according to standard procedures. Primer sets for polymerase chain reaction (PCR) were designed according to the (CGG)*n* sequence located in the first exon of the *FMR1* gene. The primer set includes the forward primer: 5′-CTCAGTCAGGCGCTCAGCTC-3′ and a reverse primer: 5′-CCATCTTCTCTTCAGCCCTGC-3′. The amplifications were performed using GeneAmp PCR system 9700 (Applied Biosystems, Foster City, USA) with the following profile: an initial step 95°C for 4 min; 40 cycles at 96°C for 30 s, 62°C for 30 s, 72°C for 1 min; and a final extension step at 72°C for 10 min. Following the amplification, the PCR products were confirmed and separated by electrophoresis on 2% agarose gel and purified using the Qiagen Gel Extraction Kit (Qiagen, Valencia, USA). Forty ng of purified PCR product was used for direct sequencing to identify the specific mutation present using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA) as described by the manufacturer.

Results

The analysis of CGG repeats was performed using PCR and sequencing analysis from the 78 males. The twenty-nine repeats (n = 55, 70.5%) was the most common length of the repeats, while the second most common was 30 repeats (n = 13, 16.7%) (figure 1). Sequencing analysis showed that most of the patients have the $(CGG)_9AGG(CGG)_9AGG(CGG)_9$ interruption pattern (presented as 9A9A9) (n = 50, 64.1%), with 10A9A9 (n = 12, 15.4%) as the second one (table 1). In addition to the normal AGG interruption in every nine or ten CGG repeats, there were six $(CGG)_6AGG$ or $AGG(CGG)_6$ patterns (7.7%) in our samples.

Keywords. fragile X syndrome; *FMR1* gene; AGG interruption; CGG repeat.

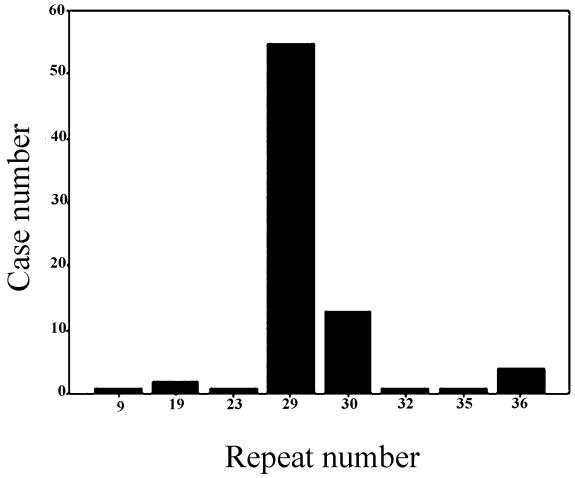


Figure 1. Distributions of the CGG repeats.

Table 1. AGG interruption patterns of *FMR1* gene.

Length	Number	Pattern
9	1	9
19	2	9A9
23	1	13A9
29	50	9A9A9
	4	9A19
	1	12A6A9
30	12	10A9A9
	1	9A10A9
32	1	10A9A11
35	1	9A8A6A9
36	3	9A9A6A9
	1	9A8A6A10

Discussion

Both the length of the repeat and interruption pattern are key issues in fragile X syndrome (Penagarikano *et al*. 2004; Glover *et al*. 2005; Entezam *et al*. 2007). In this study, we found that the 29 repeats and interruption with every nine CGG pattern are the most common figures in our population. Some studies showed ethnic differences in the length of the repeat (Crawford *et al*. 2000; Angeli *et al*. 2005). Compared to the most common repeat number, 30 repeats, in Caucasians, the majority of lengths in Asians are 29 repeats, which is similar to our report (Tzeng *et al*. 2005). We could not identify any long, pure CGG repeats in these normal individuals. This suggested that fragile X syndrome might be low in our population (Tzeng *et al*. 2005).

In our study, the interruption of the AGG was determined from the sequencing analysis. In addition, to demonstrate the most common interruption pattern, the $(CGG)_9AGG(CGG)_9AGG(CGG)_9$ interruption (presented as 9A9A9), we also found six $(CGG)_6AGG$ or $AGG(CGG)_6$ patterns (7.7%) in our samples. This special interruption concurred with previous reports that showed the insertion of six CGG in Asian populations only (Chen *et al*. 1997; Penagarikano *et al*. 2004). The major pattern of this special insertion is 9A9A6A9, which was cited in support of a possible evolutionary pathways (Chen *et al*. 1997). However, we found the pattern 12A6A9, which was not in the proposed evolutionary pathway. This might imply a complicated evolution for the generation of the insertion pattern.

In summary, we performed PCR and sequencing tests to survey the length of CGG repeats and the AGG interruption pattern of *FMR1* among the Taiwanese population. The major length of the healthy individuals is 29 repeats without any long, pure CGG repeat in normal individuals. Our data also found the special $(CGG)_6AGG$ pattern. These data reveal ethnic differences in the *FMR1* gene and implies a complicated evolution of this gene.

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