A Novel PCR Technology that Identifies AGG Interruptions in the Triplet Repeat Region of the Fragile X Gene.

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SUMMARY

- CGG triplet repeat expansions in the fragile X mental retardation gene-1 (*FMR1*) are associated with fragile X syndrome and disorders such as fragile X-associated tremor and ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency (FXPOI).
- The stability of the triplet repeat region is influenced by the presence of interrupting AGG sequences in the CGG repeat segment. However, routine methods for indentifying the AGG structure in *FMR1*, particularly in female samples, are currently lacking.
- We have developed a set of optimized and high throughput PCR reagents* that can accurately report the *FMR1* genotypes of male and female samples across the full range of clinically relevant repeat numbers, from normal to full mutation.
- The PCR reagents enabled the genotyping of DNA from 260 whole blood samples, and provided a detailed catalog of AGG interspersion patterns and resolution of the number of consecutive CGG repeats for each sample.
- Studies are ongoing to assess the implications of AGG interruptions in *FMR1* disorders.

INTRODUCTION

Fragile X syndrome is associated with the expansion of a CGG trinucleotide repeat in the 5' untranslated region (UTR) of the *FMR1* gene. These triplet repeats may be uninterrupted, or may present one or more interspersed AGG sequences near the 5' region of the repeat segment. AGG "interruptions" are thought to provide stability to the *FMR1* UTR and influence the risk of expansion in the next generation. For example, Eichler et al. suggested that most stable alleles contained one or two AGG interruptions. Alleles with 33 or fewer uninterrupted CGG repeats beyond the last AGG (toward the 3' end of the repeat) were inherited with stability, but those with 39 or more continuous CGG repeats were not. Further, the lack of any AGG sequences is a common molecular feature of each of the smallest premutations (<60 CGG) that have been found to expand to a full mutation within a single generation (Nolin et al.; Fernandez-Carvajal et al.). AGG genotypes may also be linked to FXTAS and FXPOI.

Conventional methods for identifying the positions of interrupting AGG sequences include DNA sequencing and restriction mapping with Southern blotting. Both methods are laborious, error-prone, and are not amenable to routine *FMR1* molecular genotyping. As a result, facile, streamlined, and high performing assays that can provide robust CGG repeat amplification, up to 1000 CGG repeats and beyond, and the highly specific detection of AGG sequences in all categories of *FMR1* alleles have been lacking. Here, we describe the development and testing of novel PCR reagents that can accurately assess the number of consecutive CGG repeats, and map intervening AGG sequences.

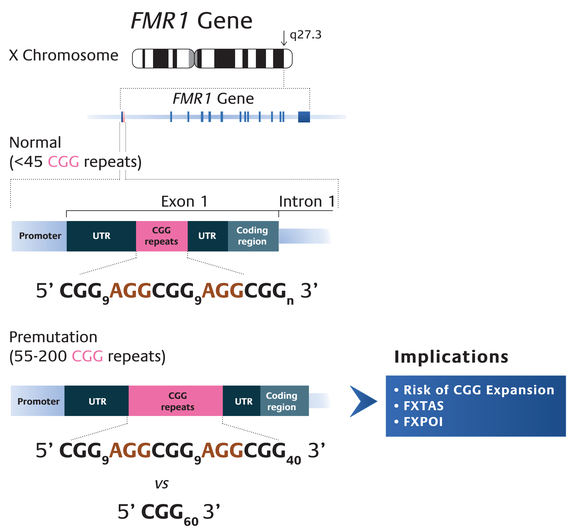

Fig. 1. *FMR1* **Gene Structure, AGG Interruptions, and Clinical Implications.** Figure adapted from Hagerman & Hagerman (2008).

*** Research Use Only. Not for Use in Diagnostic Procedures.**

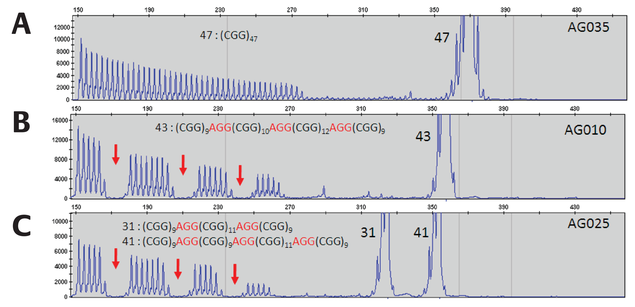

Fig. 2. The AmplideX™ CGG Repeat Primed PCR RUO Identifies Interrupting AGG Sequences in Male and Female Samples. A) Uninterrupted male sample. **B)** Male sample with 3 AGG interruptions. **C)** Female sample with overlapping AGG structures. The red arrows indicate AGG "dips" in the CE trace. The AGG sequences in most male and some female samples can be determined with this single assay. Definitive resolution of *FMR1* genotypes, however, requires additional molecular information.

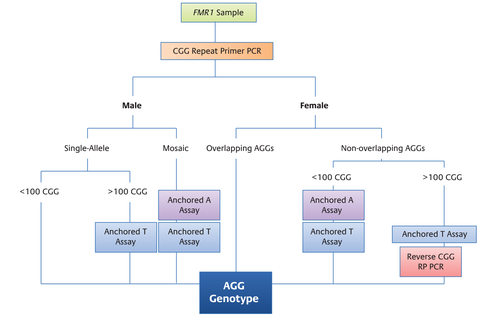

Fig. 3. Workflow for the Definitive Mapping of AGG Interruptions in *FMR1* **Samples.**

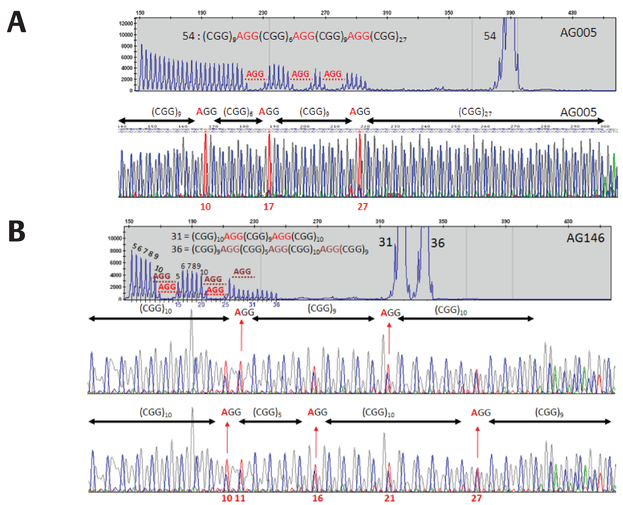

Fig. 4. Sequence Confirmation of *FMR1* **Genotypes Revealed using AGG Mapping PCR Reagents. A)** Male sample with 3 AGG interruptions. **B)** Female sample with 5 AGG interruptions, all of which are indicated by the CGG RP PCR result. Anchored A and T assays were used to resolve allele-specific AGG sequence assignments.

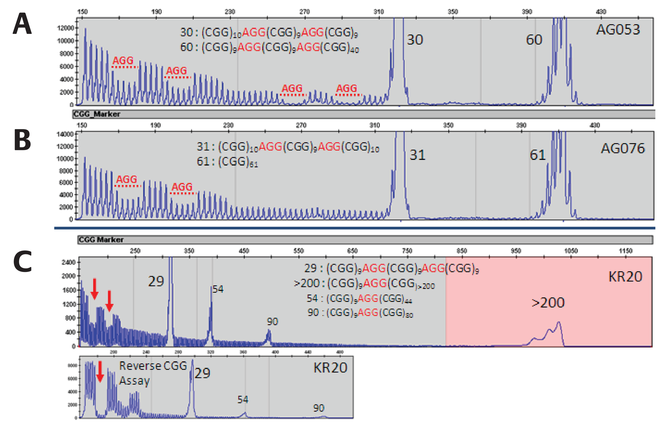

Fig. 5. *FMR1* **AGG Mapping PCR Technologies can Resolve the Genotypes of Complex Female Samples with Both Premutation and Full Mutation Expansions. A)** Female sample with AGG interruptions in both alleles. **B)** Female sample with an uninterrupted 61 CGG allele. **C)** AGG mapping in a full mutation mosaic female sample, revealing a 5' AGG in the full mutation.

Fragile X Category	#AGG					Total Alleles
	0	1	2	3	4	
Normal (<45 CGG)	17 (5.6)	73 (23.9)	170 (55.6)	45 (14.7)	1 (0.3)	306
Intermediate (45-54)	2 (5.4)	15 (40.5)	19 (51.4)	1 (2.7)	0 (0.0)	37
Premutation (55-200)	17 (36.2)	12 (25.5)	18 (38.3)	0 (0.0)	0 (0.0)	47
Full Mutation (>200)	3 (37.5)	5 (62.5)	0 (0.0)	0 (0.0)	0 (0.0)	8

Table 1. Frequency of AGG Sequence Interruptions in 398 *FMR1* **Alleles from 260 Samples.** Values in parenthesis represent the percent of all alleles for each respective category.

CONCLUSIONS

- A total of 4 unique *FMR1* PCR technologies were optimized that can identify the number and position of AGG interruptions within the CGG repeat sequence.
- AGG interspersions were identified in both normal and expanded alleles. Five of 8 full mutation samples revealed AGG interruptions.
- Of the 398 alleles interrogated with the AGG mapping PCR technologies, the majority presented 1 or 2 AGG interruptions.
- Premutation alleles were 7-fold more likely to present an uninterrupted CGG repeat tract than normal alleles, consistent with their relative instability of triplet repeat expansion.

REFERENCES

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