# Assessments of the AGG Structure in 892 Samples Reveals the Risks of Expansion for Intermediate and Small Premutation Fragile X Alleles

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## SUMMARY

- Fragile X Syndrome is characterized by an expansion of CGG triplet repeats in the 5'-untranslated region of the fragile X mental retardation (*FMR1*) gene.
- Many *FMR1* alleles contain AGG sequences that are thought to reduce the risk of repeat expansion in the next generation.
- 830 females and 62 males were analyzed for AGG status and stability on transmission.
- This cohort analysis is the largest study to date and the only study to focus predominantly on female samples.
- Risks for repeat expansion and implications for genetic counseling are presented.

## BACKGROUND

Fragile X Syndrome (FXS) is characterized by an expansion of CGG triplet repeats in the 5'-untranslated region of the fragile X mental retardation (*FMR1*) gene. Many *FMR1* alleles contain AGG sequences that are interspersed among the CGG repeats, usually in the 5' region of the repeat segment. These AGG "interruptions" are thought to confer DNA stability, and have been suggested to reduce the risk of expansion in the next generation (Eichler 1994). Further, the lack of any AGG sequences is a common molecular feature of each of the smallest premutations (<60 CGGs) that have been found to expand to a full mutation within a single generation (Nolin 2003 and Fernandez-Caravjal 2009).

Existing methods to identify AGG sequences, such as sequencing and restriction enzyme mapping, are laborious and are not routinely performed. High throughput technologies that can accurately detect AGG interspersions are needed to enable more comprehensive molecular profiling of the *FMR1* gene. Knowledge of AGG structure may improve risk stratification for women with intermediate and small premutation alleles.

## METHODOLOGY

Four institutions submitted 892 samples to Asuragen for AGG mapping analysis. All samples were processed with AmplideX™ CGG Repeat Primed (RP) PCR* to size alleles and to infer presence of AGG sequences. Two additional novel PCRs were used to definitively resolve the AGG location on each allele. The results were tabulated according to sample pedigree, repeat length and allele location of each AGG. Within parent to child transmissions, alleles ≥ 1 CGG difference were flagged as unstable. To date, 1628 alleles and 216 transmissions have been fully annotated. Results for these annotated samples are presented here.

### The AmplideX™ CGG Repeat Primed PCR Identifies Interrupting AGG Sequences Important to Risk Stratification in FXS Testing

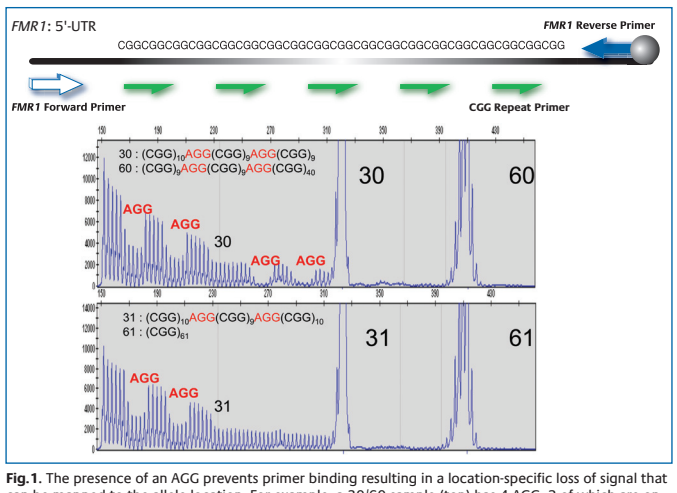


**Fig.1**. The presence of an AGG prevents primer binding resulting in a location-specific loss of signal that can be mapped to the allele location. For example, a 30/60 sample (top) has 4 AGG, 2 of which are on the 60 CGG allele, and a 30/61 sample (bottom) has no AGG on the longer allele. *Note that definitive resolution of AGG genotypes in female samples requires additional molecular information (Chen 2010 and Latham 2010).*

### AGG Mapping is Concordant with DNA Sequencing and Provides Repeat Resolution of 1 CGG

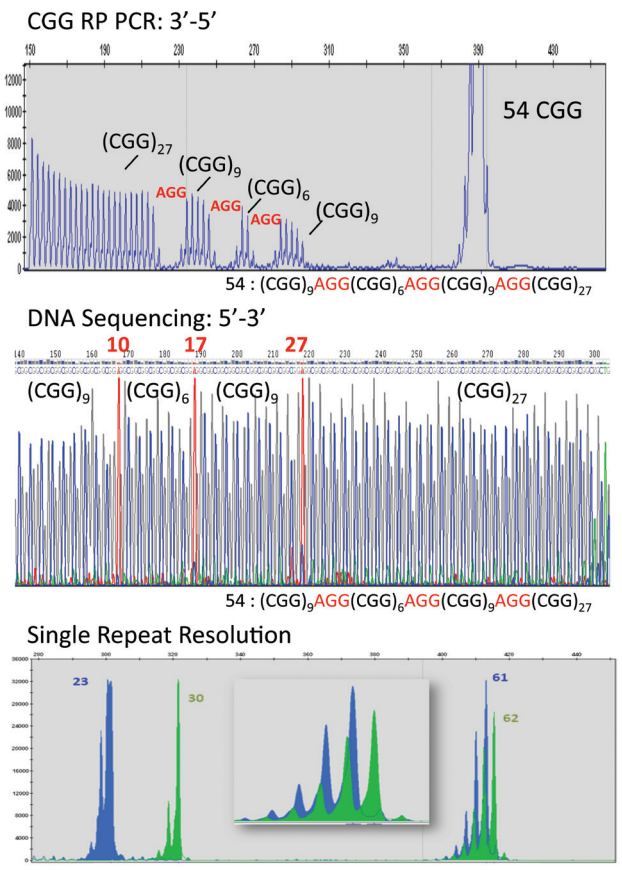


**Fig. 2.** The sequence context of each AGG can be elucidated by peak counting of CGG RP PCR products in concordance with DNA sequencing. Sizing by CE identifies 1 CGG repeat differences in transmitted alleles. Out of 18 parent-child alleles differing by 1 CGG, the average difference was 0.9 ± 0.2 CGG compared to <0.3 CGG from multi-day and operator testing of 5 alleles ranging from 20-119 CGGs (54 replicates of each allele).

### The Number of Interspersed AGG Decreases with Increasing Repeat Length

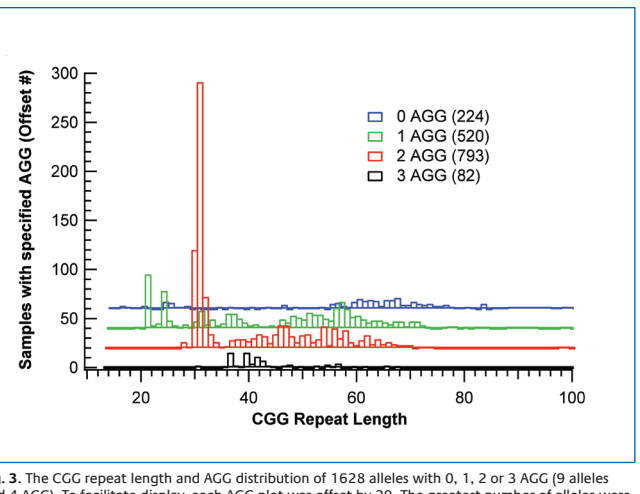


**Fig. 3.** The CGG repeat length and AGG distribution of 1628 alleles with 0, 1, 2 or 3 AGG (9 alleles had 4 AGG). To facilitate display, each AGG plot was offset by 20. The greatest number of alleles were 28-32 CGGs with 2 AGG. Longer alleles were associated with 0 or 1 AGG.

### The Risk for Expansion Increases as a Function of 3'-continuous CGG Repeat Length

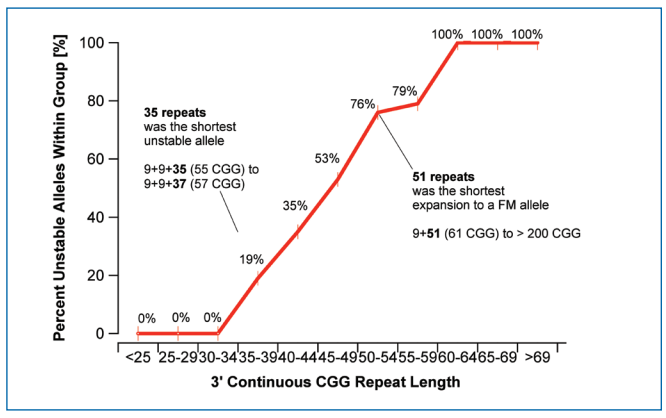


**Fig. 4**. This summary of 216 transmitted alleles shows the increasing risk of expansion with the 3'-continuous CGG repeat length. The shortest unstable 3'-length was 35 CGGs and all alleles greater than 60 uninterrupted CGGs were unstable. No alleles were associated with gain of AGG; only 3 alleles were associated with loss of AGG.

### The Number of AGGs Can Affect the Risk and Magnitude of CGG Repeat Expansion for Equivalently Sized Alleles

**Effect of AGG structure on 55 repeat allele transmissions**

| Number AGG | Unstable Alleles (Number/Total) | Repeat Increase (Average) |
|---|---|---|
| 0 | 7/7 | 4-14 (9) |
| 1 | 6/16 | 1-3 (2) |
| 2 | 1/12 | 2 (2) |
| 3 | 0/1 | 0 (0) |

**Table 1**. An analysis of 36 alleles with 55 CGG repeats shows a difference in risk of expansion and magnitude of that expansion associated with AGG.

## CONCLUSIONS

- A combination of commercially available and novel *FMR1* PCR research technologies were used to assess AGG structure in 892 samples representing 216 transmissions.
- Instability was overwhelmingly characterized by an increase in CGG repeat length on the 3'-end.
- The uninterrupted CGG length affected the risk of expansion in equally sized alleles.
- Current professional guidelines lack resolution of alleles at-risk for expansion.
- Knowledge of AGG structure in intermediate and premutation alleles can refine risk stratification and improve genetic counseling.

## References

Eichler EE et al. Nat Genet. 1994 Sep;8(1):88-94; Nolin SL et al. J Hum Genet. 2003 Feb;72(2):454-64.; Fernandez-Carvajal I et al. J Mol Diagn. 2009 Jul;11(4):306-10; Chen L et al. J Mol Diagn. 2010 Sep;12(5):589-600; Latham GJ et al. Association of Molecular Pathology, San Jose, CA, Nov. 2010.

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Disclosure: AH, RS, SS, and GJL are employees of Asuragen, Inc.
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