Personalizing Risk for *FMR1* Premutation Carriers: Insights from over 8000 AGG Test Results

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Summary

- AGG interruption sequences within the *FMR1* gene assist in providing stability of the CGG repeat region.
- Asuragen's Xpansion Interpreter® service offers routine testing for AGG with volumes increasing yearly.
- We report our experience with 8,634 clinical samples submitted for AGG interruption testing and case study examples of post-test risks for fragile X full mutation expansion.

Introduction

PCR technologies that genotype the CGG repeat region of the *FMR1* gene enable precise mapping of total repeat length and interrupting AGG sequences, as compared to older Southern blotting methodologies. AGG interruptions stabilize the CGG repeat region and mitigate transmission expansion.[1] Two studies have demonstrated that AGG mapping provides more accurate risk estimates for the likelihood of expansion in offspring.[2,3] The authors concluded that assessment for AGG interruptions is important for accurate risk assessment in counseling fragile X carriers.[2,3] Since publication in 2014, we have seen a marked increase in samples submitted for reflex testing. We report our experience with AGG interruption testing on over 8,000 clinical samples submitted to the Asuragen Clinical Laboratory to understand better the clinical utility of this test.

Methods

Xpansion Interpreter uses Repeat-Primed Polymerase Chain Reaction (RP-PCR) amplification of the 5'-untranslated region of the *FMR1* gene and additional assays to determine both the number and sequence context of AGGs within the CGG repeat tract. Over the past four years, 8,830 clinical samples were evaluated in Asuragen's clinical laboratory. Sizing accuracy within each run was achieved using established AmplideX® *FMR1* controls. Individualized post-test risks for expansion to a fragile X full mutation were derived from regression modeling performed on AGG interruption data from a separate cohort of mother-to-child *FMR1* transmissions comprised of 597 *FMR1* transmissions from women with 45-69 CGG repeats[3,4] plus an additional 321 maternal transmissions from women with 70-90 CGG repeats.[4] Pre-test risks for transmission of a fragile X full mutation were obtained from Nolin et al., 2003[5], represented in the 2012 NSGC Practice Guidelines[6] and 2010 ACOG Committee Opinion.[7] Samples that were found to be outside of these ranges (less than 45 or greater than 90), samples containing minor expanded alleles, and male samples were excluded from this analysis.

Results

8,830 clinical samples were submitted for AGG interruption testing, representing an average 80% increase year over year from 2014 to 2018 (Figure 1). Of these, 196 contained CGG repeat status outside of the range accepted for Xpansion Interpreter testing. For the remaining 8,634 specimens, results included 3,362 premutation carriers (38.9%), 5,185 intermediate carriers (60.1%), and 87 dual carriers with 2 intermediate, 2 premutation, or 1 intermediate plus 1 premutation (1.0%). Unexpected results included a mosaic case with a full mutation and premutation and 7 cases with an insertion of extra DNA within the CGG repeat region. 91.8% of the samples tested contained at least 1 AGG interruption (Figure 2). The refined risk applied to the results of 3,379 premutation cases as shown in Figure 3 supports the importance of AGG interruption reflex testing as 85% had a lower risk of expansion, 12% had higher risk, and only 3% remained unchanged (within 1% of original risk). Examples of how patients and providers used AGG testing to make medical or family decisions are also presented as case examples.

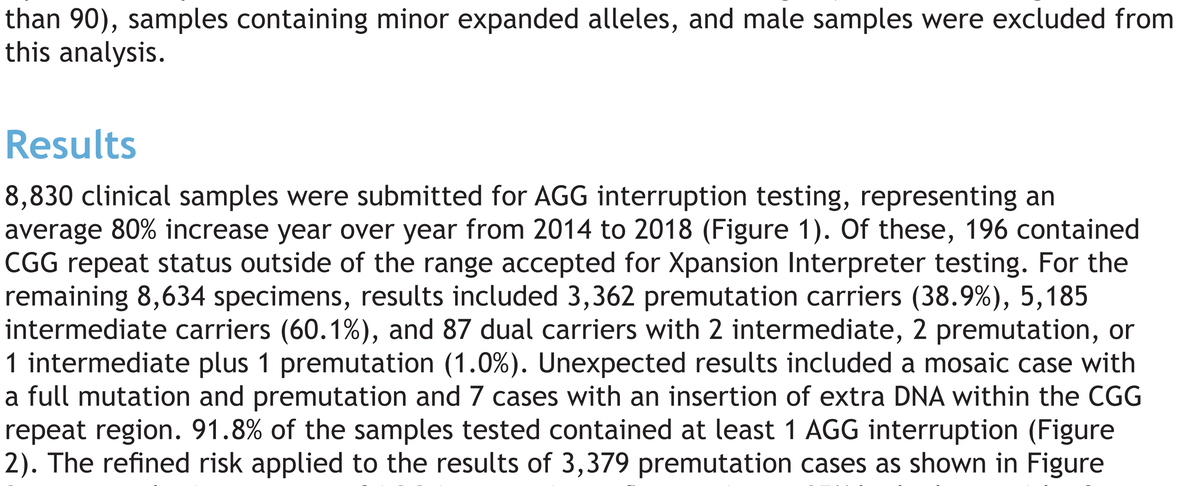

Figure 1. Clinical Testing Volumes have Increased Significantly. Xpansion Interpreter testing volume for specimens within the 45-90 CGG repeat range have increased on average 80% year over year.

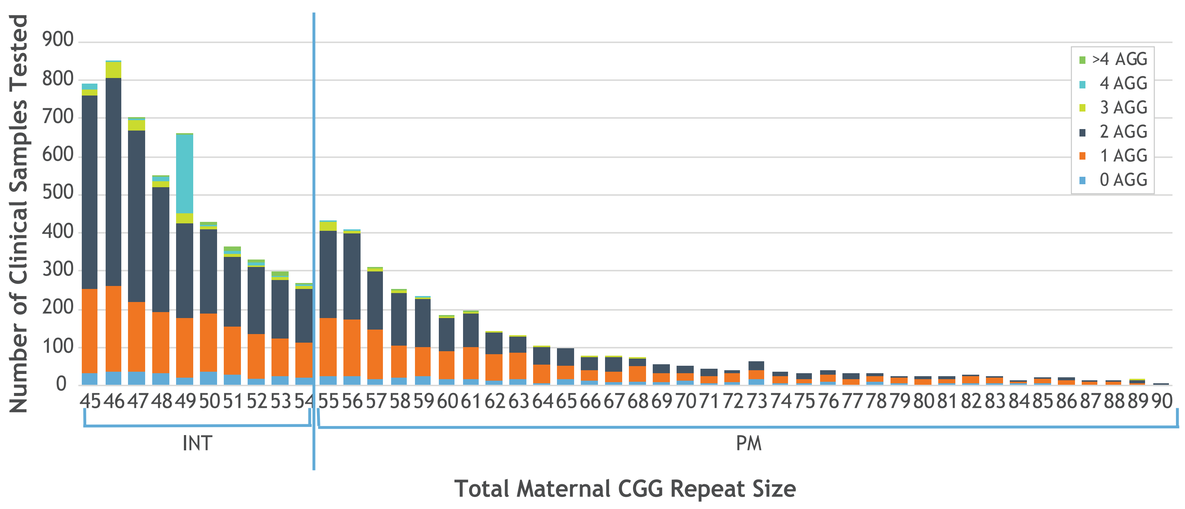

Figure 2. Distribution of AGG Interruptions and Maternal Repeat Length in 8,634 Clinical Samples Tested. 5,185 represented intermediate carriers and 3,362 represented premutation carriers. For the 87 dual carriers, only the longer allele is represented. The presence of 0, 1, 2, 3, 4, or >4 AGG interruptions in these patient samples are shown. INT=Intermediate carrier, PM=Premutation carrier

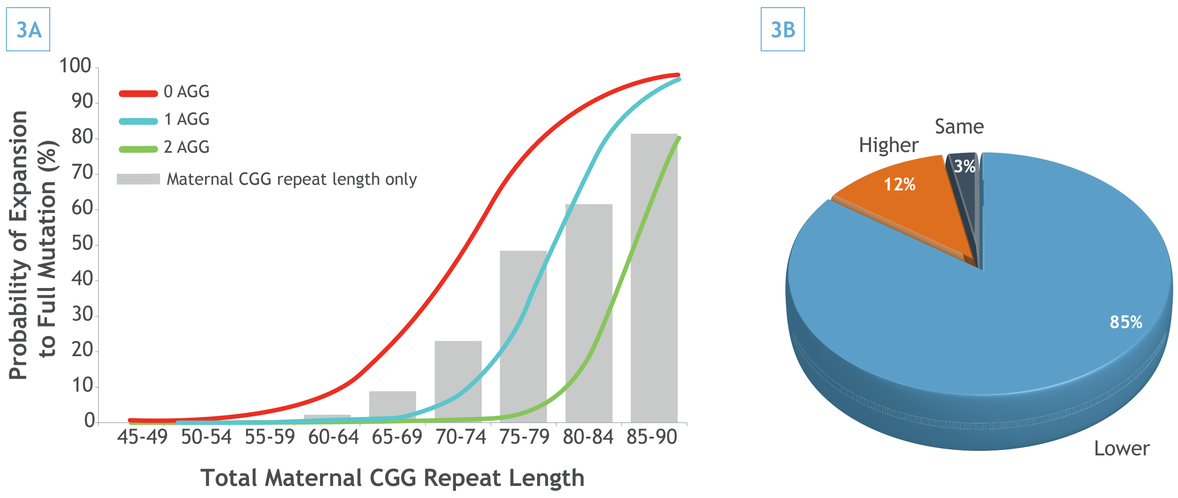

Figure 3. Traditional and Refined Risk Estimates for Fragile X Full Mutation Expansions with Distribution of Clinical Cases Evaluated by the Diagnostic Laboratory. The data shown in panel **(A)** represent a combination of published results (Nolin, et al., 2013 and Nolin, et al., 2015). The graph illustrates the probability of expansion to a fragile X full mutation in the next generation based on the total number of maternal CGG repeats alone (gray bars) and as further refined by the number of AGG interruptions present (colored lines). In panel **(B)** we show the distribution of 3,379 clinical premutation cases and whether the Xpansion Interpreter AGG interruption report provided a higher, lower, or similar risk of expansion.

Case Examples

CASE 1

Female patient with 68 CGG repeats and a family history of fragile X syndrome decided to have AGG testing to refine further her risk prior to her next pregnancy. AGG interruption testing found 68 CGG repeats and zero AGG interruptions, which provided an increased risk estimate to 27% (95% Confidence Interval: 19%-36%) for full mutation expansion should the expanded *FMR1* allele be passed to a pregnancy. The patient decided to explore alternative reproductive options.

CASE 2

Female patient found out she had 59 CGG repeats as part of laboratory work-up for IVF treatments due to unrelated reasons. She was quoted a 3.7% risk for expansion to a full mutation should the expanded FMR1 allele be passed to offspring, which she perceived as too high. She wondered if she should be considering PGD. The patient underwent AGG testing where she was found to have 1 AGG interruption, resulting in a <1% chance of expansion. This was reassuring to the patient as she was comforted knowing that her allele was much more stable than previously thought. This testing provided closure for the patient.

Conclusions

AGG testing is utilized by fragile X carriers and their genetics, infertility, and primary care providers to obtain a better understanding of the risk estimates for expansion of their *FMR1* gene, and to make family planning and reproductive decisions.

- Volume of AGG reflex testing has increased an average of 80% annually over the last four years as clinicians are finding increased value in counseling patients who are carriers.
- Post-test risks for *FMR1* allele expansion to a fragile X full mutation following AGG testing changed in nearly all cases compared to pre-test risks.
- Patients are incorporating information learned from AGG interruption testing into their decisions regarding reproductive options and family planning.

References

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