

Background

Fragile X syndrome is caused by a mutation in the Fragile X Mental Retardation 1 (*FMR1*) gene and is inherited in an X-linked manner. All copies of the *FMR1* gene have a triplet CGG repeat in the 5' UTR untranslated region of *FMR1*. When expansion of this region exceeds a certain threshold (>200 repeats), it causes the gene to become inactive, resulting in fragile X syndrome.

A premutation (PM) result is between 55 and 200 CGG repeats. Women who carry a PM are not affected with fragile X syndrome, but they are at risk to have a child with fragile X syndrome. PM sizes can be unstable when passed from a woman to her child and the risk of a PM to expand to a full mutation is correlated with CGG repeat size.

The number of AGG interruptions within the CGG repeat sequence impacts the likelihood that a CGG repeat will expand when passed from mother to child. AGG interruption testing can further define the risk of expansion as the presence of ≥1 AGGs may reduce, but does not eliminate, the chance of expansion to a full mutation in female carriers with PM in the 55-90 CGG range. AGG testing is not indicated for PM carriers with CGG repeats greater than 90 as it has not shown to impact the risk of expansion.

Reproductive options exist for female PM carriers to reduce the risk of having an affected child. These options include prenatal diagnosis during pregnancy, in vitro fertilization (IVF) with an egg donor, as well as IVF with Preimplantation Genetic Diagnosis (PGD). Prior to undergoing PGD, a setup is required which may take several weeks and testing of additional family members may be necessary. Carriers who are identified prior to conception have the most reproductive options available.

Objective

To present our initial experience with AGG reflex testing and preconception reproductive decision-making in fragile X carriers identified in the infertility setting.

Materials & Methods

Fragile X screening was performed using triplet repeat PCR analysis. AGG reflex testing was performed on select PM carriers with 55-90 CGG repeats. This testing was performed at Asuragen Clinical Laboratory (Austin, Texas) and risk revisions were provided on their reports. We re-contacted patients and/or referring providers to determine what reproductive decisions were made after the patients were provided the modified risk revisions post-AGG analysis.

Figure 1. Breakdown of Allele Sizes of Female Fragile X Carriers

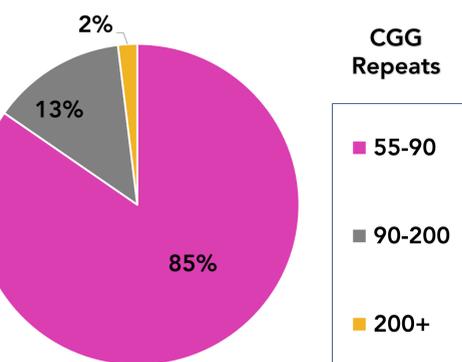


Figure 2. AGG Results Breakdown

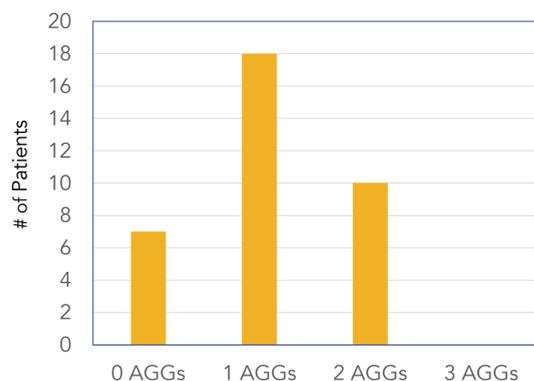


Figure 3. Percentage of Patients in Refined Expansion Risk Categories

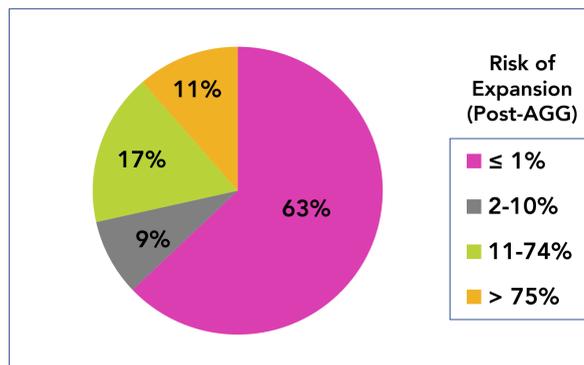
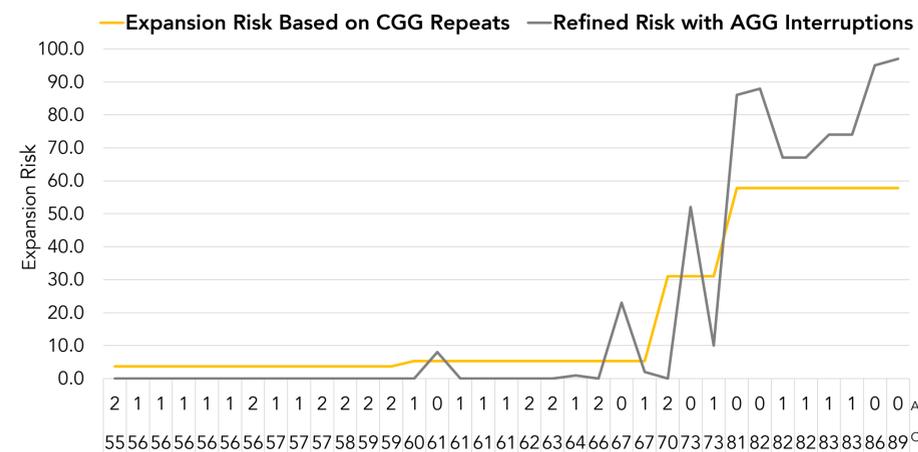


Table 1. Patient Results and Refined Expansion Risks

Patient	CGG Repeats	Number of AGG Interruptions	Initial Expansion Risk (CGG Only)*	Refined Expansion Risk (Post-AGG)
#1	55	2 AGG	3.7%	<1%
#2	56	1 AGG	3.7%	<1%
#3	56	1 AGG	3.7%	<1%
#4	56	1 AGG	3.7%	<1%
#5	56	1 AGG	3.7%	<1%
#6	56	1 AGG	3.7%	<1%
#7	56	2 AGG	3.7%	<1%
#8	57	1 AGG	3.7%	<1%
#9	57	1 AGG	3.7%	<1%
#10	57	2 AGG	3.7%	<1%
#11	58	2 AGG	3.7%	<1%
#12	59	2 AGG	3.7%	<1%
#13	59	2 AGG	3.7%	<1%
#14	60	1 AGG	5.3%	<1%
#15	61	0 AGG	5.3%	8%
#16	61	1 AGG	5.3%	<1%
#17	61	1 AGG	5.3%	<1%
#18	61	1 AGG	5.3%	<1%
#19	62	2 AGG	5.3%	<1%
#20	63	2 AGG	5.3%	<1%
#21	64	1 AGG	5.3%	1%
#22	66	2 AGG	5.3%	<1%
#23	67	0 AGG	5.3%	23%
#24	67	1 AGG	5.3%	2%
#25	70	2 AGG	31.1%	<1%
#26	73	0 AGG	31.1%	52%
#27	73	1 AGG	31.1%	10%
#28	81	0 AGG	57.8%	86%
#29	82	0 AGG	57.8%	88%
#30	82	1 AGG	57.8%	67%
#31	82	1 AGG	57.8%	67%
#32	83	1 AGG	57.8%	74%
#33	83	1 AGG	57.8%	74%
#34	86	0 AGG	57.8%	95%
#35	89	0 AGG	57.8%	97%

* Initial expansion risk from Nolin et al. Am J Med Genet 2003. 72(2):p454-64.

Figure 4. Comparison of Patient Expansion Risk % Based on CGG Repeats versus Refined Risk % with AGG Interruptions



Results

In this study, 82,135 females from US fertility clinics underwent routine fragile X syndrome carrier screening by CGG analysis. Of those tested, 365 patients were identified as having ≥55 CGG repeats, giving an observed carrier frequency of ~ 1 in 225. Of the 365 carriers, 358 were in the PM range and 7 were full mutation carriers (Figure 1). The majority (86.3%) of PM carriers detected had repeats in the range of 55 and 90. 35 patients had follow-up AGG testing in order to refine their risk of expansion. Table 1 summarizes the CGG and AGG results of these 35 patients, including their adjusted risk estimates.

Figure 2 shows the AGG result breakdown, 28 of 35 patients were found to have 1-2 AGGs, reducing their risk of expansion to a full mutation, while 7 patients had 0 AGGs, putting them at increased risk. We did not find any patients with 3 AGGs. The majority of patients (62.9%) had a final risk of expansion of 1% or less. Only 4 patients (11.4%) had a final risk of expansion >75%. Figure 3 shows the percentage of patients in different risk expansion categories after AGG testing. Figure 4 shows the comparison of original expansion risks based on CGG repeats versus refined expansion risks once AGG interruption data was known. Only 1 patient (#23) with a starting risk of ≤5.3% had their adjusted expansion risk increase more than 5%. Additionally, in our small dataset, none of the patients with an initial expansion risk of >50% had a decrease in their refined risk numbers.

Through follow-up, we learned that:

- ❖ Many patients did NOT alter their reproductive decisions between the time they were initially told of their fragile X carrier status and when they received their enhanced risk assessment.
- ❖ Those patients who were already planning on doing PGD, did NOT change their reproductive decision even when their refined risk was less than 1%.

Patient #32 intended to do PGD, however, she had Primary Ovarian Insufficiency (POI) and didn't make any embryos (with multiple IVF cycles) and therefore was unable to undergo PGD.

Patient #3 initially opted to start with PGS to rule out aneuploidy and then to re-biopsy any euploid embryos for PGD since she did not want to delay her IVF cycle for the time required for the PGD setup. She continued to want PGD for fragile X even with a revised risk of expansion of <1%.

Patient #17 had all along decided against PGD. It was reported that she was reassured about her decision when informed that her revised risk of expansion was <1%.

Conclusions

AGG interruption testing can further modify the expansion risk for female fragile X PM carriers with 55-90 CGG repeats. In our study, approximately 0.38% of females tested were eligible for AGG testing. For these women, their AGG status has the potential to significantly modify their risk of repeat expansion and therefore their risk to have an affected child.

Our initial experience demonstrates that female PM carriers pursuing fertility treatments may not alter their reproductive decisions based on the refined risk assessment provided by AGG analysis. Additionally, even when provided with a <1% risk of expansion, patients often still opted to pursue PGD. However, in this cohort, AGG testing did not have a drastic impact on expansion risk for most patients. Assessment of decision-making in women with a larger risk reduction may provide more insight. Additional factors that may impact decision-making include having previous affected children, having POI/infertility, as well as the indication for doing carrier screening (egg donor or egg banking vs. actively pursuing pregnancy).

As a lab that now reports AGG testing on all appropriate fragile X PM samples, we will continue to track patient decisions. Many of our patients received the information sequentially, and future directions for research include assessing reproductive decision-making when patients are provided both CGG and AGG information together (versus sequentially) in both prenatal and preconception (non-infertile) populations. More detailed follow-up for patients being provided this information and better education for patients and their providers about the meaning of these results is still needed.

Figure 5. Expansion Risk of Patients Desiring Preimplantation Genetic Testing

