All-in-one solution for single gene disorders with repeat elements

Fragile X Syndrome

Background

Fragile X syndrome (FXS) is considered the most common inherited cause of intellectual disability and the second most prevalent cause after Down syndrome. [1] It is caused by mutations in the *FMR1* gene located on the X chromosome. Female patients generally exhibit a less severe phenotype than male patients. More than 99% of FXS patients have abnormal methylation resulting from the overexpansion of CGG repeats within the *FMR1* gene, while a small proportion of FXS patients have defects in *FMR1* gene function due to other variants such as deletions and SNVs. The categories of CGG repeat numbers and associated clinical disorders are as follows:

Categories	Number of CGG repeat	Clinical significance
Normal	5 - 44	--
Intermediate	45 - 54	--
Premutation	55 - 200	Increased risk for fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency (FXPOI); Women carriers are at risk of having FXS children
Full mutation	>200	FXS

Individuals with a premutation allele can transmit a full mutation allele to the next generation due to the dynamic expansion of CGG repeats during germline transmission. Additionally, the presence of AGG interruptions within CGG repeats can reduce the likelihood of expansion to a full mutation [2], which is important information for genetic counseling but is often overlooked under carrier screening.

The presence of **AGG interruptions** within CGG repeats can **reduce** the likelihood of **expansion to a full mutation**

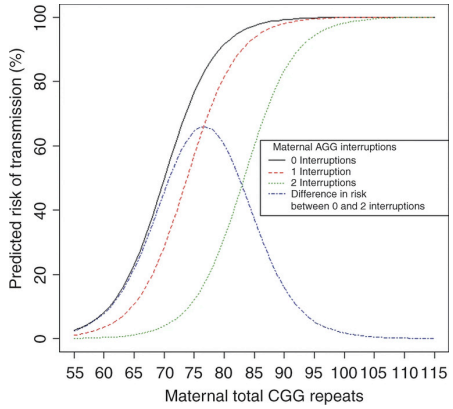
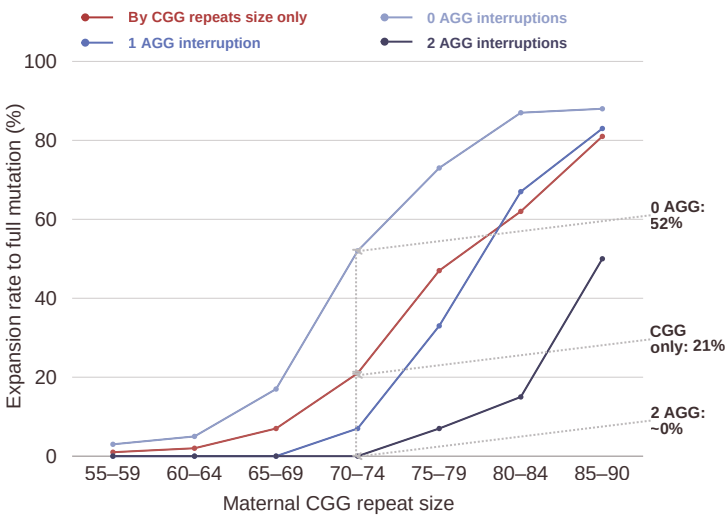
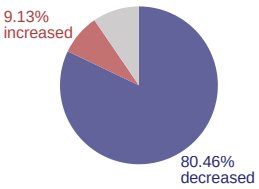


Figure adopted from reference [2]

Rate of full mutation expansions sorted by repeat size and number of AGG interruption



Information derived from reference [3]



Another study found that **incorporating AGG interruption** analysis into standard premutation screening **modified risk in 83.2% of premutation carriers**, with 80.46% showing decreased risk and 9.13% increased risk [4]

Conventional molecular assay methods typically focus on determining the number of CGG repeats and/or the methylation status of the *FMR1* gene only. However, Southern blot analysis has low resolution for CGG repeat numbers, and PCR-based assays struggle to accurately determine exact high repeat numbers. Additionally, these conventional methods are not suitable for detecting other *FMR1* gene variants, and are limited or unable to identify AGG interruptions.

FXS-specific PCR-based assay is not applicable for:

- Definitely distinguishing between high repeat numbers in permutation and full mutation
- AGG interruptions
- Mutations in repeat-flanking regions that prevent primer annealing

Comprehensive Analysis of Fragile X Syndrome

Comprehensive Analysis of Fragile X Syndrome (CAFXS)

covers in advance with the factors which are often being unnoticed

Powered by Single Molecule Real-Time (SMRT) sequencing, CAFXS offers a cost-effective and advanced solution for FXS analysis, addressing previously overlooked factors. It offers simultaneous detection of CGG repeat numbers, AGG interruptions, and associated variants often missed by PCR-based assays, providing reliable results to support informed decision-making and resolve challenging cases.

Type of variants		Panel	
5′ untranslated region (UTR)	CGG repeat numbers	Basic	Comprehensive
	AGG interruption numbers and positions		
Exon 1	Deletions		

Technology: Single molecule real-time (SMRT) sequencing
Platform: PacBio Vega, Sequel II, and Sequel IIe system
Sample type: Dried blood spot (DBS), gDNA, blood, buccal swab, and amniotic fluid
Operation hour: 67 hours
(starting from sample preparation to report generation)

Offers advanced risk predictions for premutation carriers

Provides detailed AGG interruption analysis, enabling more accurate genetic counseling and improved risk assessment

Investigates uncommon cases

Detects deletions often missed by PCR-based assays due to flanking region challenges, effectively resolving these complex cases with CAFXS

Determines a more precise number of CGG repeats

Accurately distinguishes CGG repeat numbers across a wide range of repeat expansions, ensuring precise and reliable results

Superior sensitivity for mosaicism

Offers 2–4 times greater sensitivity than TP-PCR, detecting mosaic alleles as low as 0.5% mosaicism [5]

	TP-PCR + CE	Southern blot analysis	CAFXS
CGG repeat numbers	At risk of misinterpreted premutations and full mutations	Low resolution	**Up to 940 repeats [5]**
Heterozygous vs Homozygous	✓	×	✓
AGG interruptions	Limited	×	✓
Methylation	×	✓	×

Performance backed by published data

In two seperated studies, CAFXS demonstrated/identified:

- all CGG expansions for 238 high-risk clinical samples [6]
- 3 cases from the same family with deletions in exon 1 or the upstream region of CGG repeats [6]
- all AGG interruptions in a total 62 clinical samples, including those with mosaic alleles [5]
- additional 3 samples with mosaic permutation alleles were missed by TP-PCR and Southern blot [5]

References: [1] Saldarriaga W, Tassone F, González-Teshima LY, Forero-Forero JV, Ayala-Zapata S, Hagerman R. Fragile X syndrome. *Colomb Med (Cali)*. 2014;45(4):190-198. [2] Yrigollen CM, Durbin-Johnson B, Gane L, Nelson DL, Hagerman R, Hagerman PJ, Tassone F. AGG interruptions within the maternal *FMR1* gene reduce the risk of offspring with fragile X syndrome. *Genet Med*. 2012 Aug;14(8):729-36. [3] Nolin SL, Glicksman A, Ersalesi N, et al. Fragile X full mutation expansions are inhibited by one or more AGG interruptions in premutation carriers. *Genet Med*. 2015;17(5):358-364. [4] Westemeyer M, Saucier J, Wallace J, et al. Clinical experience with carrier screening in a general population: support for a comprehensive pan-ethnic approach . *Genet Med*. 2020;22(8):1320-1328. [5] Hou F, Mao A, Shan S, et al. Evaluating the clinical utility of a long-read sequencing-based approach in genetic testing of fragile-X syndrome. *Clin Chim Acta*. 2023;551:117614. [6] Liang Q, Liu Y, Liu Y, et al. Comprehensive Analysis of Fragile X Syndrome: Full Characterization of the *FMR1* Locus by Long-Read Sequencing. *Clin Chem*. 2022;68(12):1529-1540.
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