

FRAXE molecular diagnosis in individuals referred for FRAXA screening

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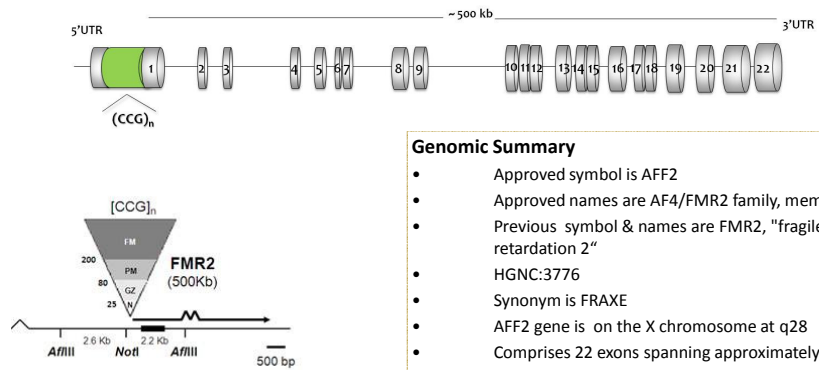
Introduction

FRAXE mental retardation is a form of mild to moderate intellectual disability generally associated with learning difficulties, communication deficits, attention problems, hyperactivity and autistic behavior. FRAXE (AFF2/ FMR2 gene), a folate-sensitive fragile site in Xq28 ~600 kb distal to the FRAXA (FMR1 gene) site, is the most common form of inherited mental retardation. Molecular characterization revealed that individuals expressing FRAXE had amplifications of a CCG repeat adjacent to a CpG island. Normal individuals showed 4–39 copies of the polymorphic FRAXE CCG repeat, while individuals expressing the fragile site had >200 copies and their CpG island was fully methylated. These findings are similar to those found for folate-sensitive fragile X site FRAXA.

Reports of FRAXE full expansions and pre-mutations are rarely documented. In this respect, it has been very difficult to determine to what extent the alleles, with CCG repeats in the range of 36 to 199, have a pathogenic effect. Intellectually disabled individuals are primarily referred for FRAXA screening and individuals who are negative for FRAXA are possible candidates for FRAXE screening. Traditionally in some laboratories AFF2 molecular analysis is performed by PCR; it is known that CCG repeats in the range of ~80 and above are not reliably amplified. We embarked on an effort to supplement our PCR analysis by Southern blot and cloned a segment of the AFF2 gene that can be used by appropriate labeling as a probe to determine expansion of the CCG repeats in the AFF2 gene.

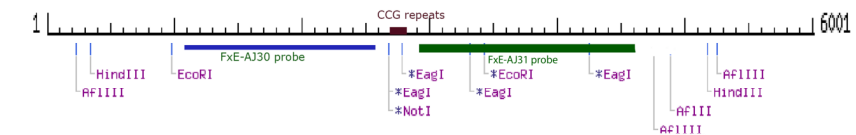
Conclusion

We have developed a probe to be used for Southern blot analysis that reliably detects the AFF2 CCG triple repeat amplification. We present data of AFF2 molecular analysis in a subpopulation of 5,000 individuals referred for FRAXA screening. The presence of pre-mutated and fully expanded alleles in either gender, were confirmed by Southern blot analysis, which also enabled exclusion of methylation or repeat number mosaics as well as PCR failure. We recommend the use of this probe as suitable for genotyping of pre-mutations, full mutations, and mosaics specifically for individuals presented for FRAXA screening with negative results to determine FRAXE status.



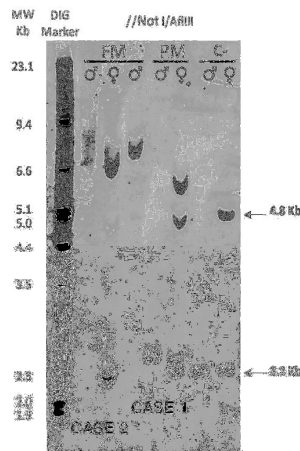
Genomic Summary

- Approved symbol is AFF2
- Approved names are AF4/FMR2 family, member 2
- Previous symbol & names are FMR2, "fragile X mental retardation 2"
- HGNC:3776
- Synonym is FRAXE
- AFF2 gene is on the X chromosome at q28
- Comprises 22 exons spanning approximately 500 kb.
- The AFF2 CCG repetitive region is located in the 5'-UTR



FraxE-AJ28 6 kb

all approximate sizes refer to restriction table



Probe Construction & Cloning

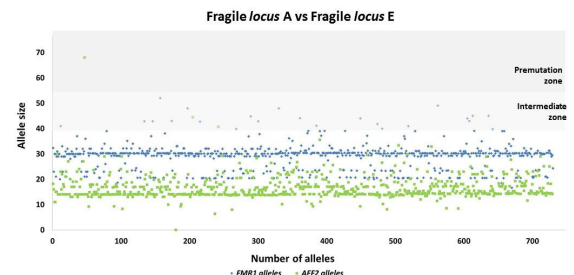
- Sequence identified upstream and downstream of the AFF2 CCG repeat region
- Synthetic constructs designed based on restriction pattern fragment for cloning of probes

FRAXE Restriction Pattern Fragments & CCG Repeats

- gDNA double digestion with NotI and AflIII restriction enzymes
- Normal ~2.2 kb for the unmethylated allele (active X) and ~4.8kb for the methylated allele (inactive X). 4-39 CCG repeats.
- Premutation alleles 2.2kb+ and 4.8kb+ fragments. 50-200 CCG repeats
- Full mutation range more than 200 repeats with fully methylated CpG island. 0.8 kb + expansion of fragments.

Triple Repeat Variation in FRAXA and FRAXE

- Triple repeat size content in FMR1 gene is independent of variations in AFF2 triple repeats.
- Most frequent combination content occurrence of 30 repeat-sized alleles for FMR1 and 14 repeat-sized alleles for AFF2.



Southern Blot Restriction Pattern & Fragments

- Lane 1 – DNA Molecular Weight Marker
- Lanes 2, 3, and 4 are from the CASE 2 with a full mutation allele (FM) with ~500 CCG, mother, and brother.
- Lanes 5 and 6 represent results from the CASE 1 showing a "pure" pre-mutation with ~68CCGs and his mother.
- Lanes 7 and 8 are the normal controls (C-).

Probe Availability

Contact Gene Link, Inc.

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www.genelink.com

Relationship(s) to Disclose: Yes

Gene Link, Inc (3)



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