Diagnosing Fragile X syndrome by DNA methylation array

Fragile X syndrome (FXS) is the most common genetic condition which causes a range of developmental problems and intellectual disability in males. It is caused by a mutation in the fragile X mental retardation gene (*FMR1*), which is located on chromosome X. Disease is caused by an abnormal expansion of the CGG trinucleotide repeat located in this gene's promoter. Normal FMR1 gene can have up to 44 CGG repeats, while premutation form have 55-200 repeats, and full mutation has more than 200 repeats. In people with fragile X syndrome the full CGG expansion results in DNA methylation of the gene promoter which turns off the *FMR1* gene, lading to FXS in these patients.

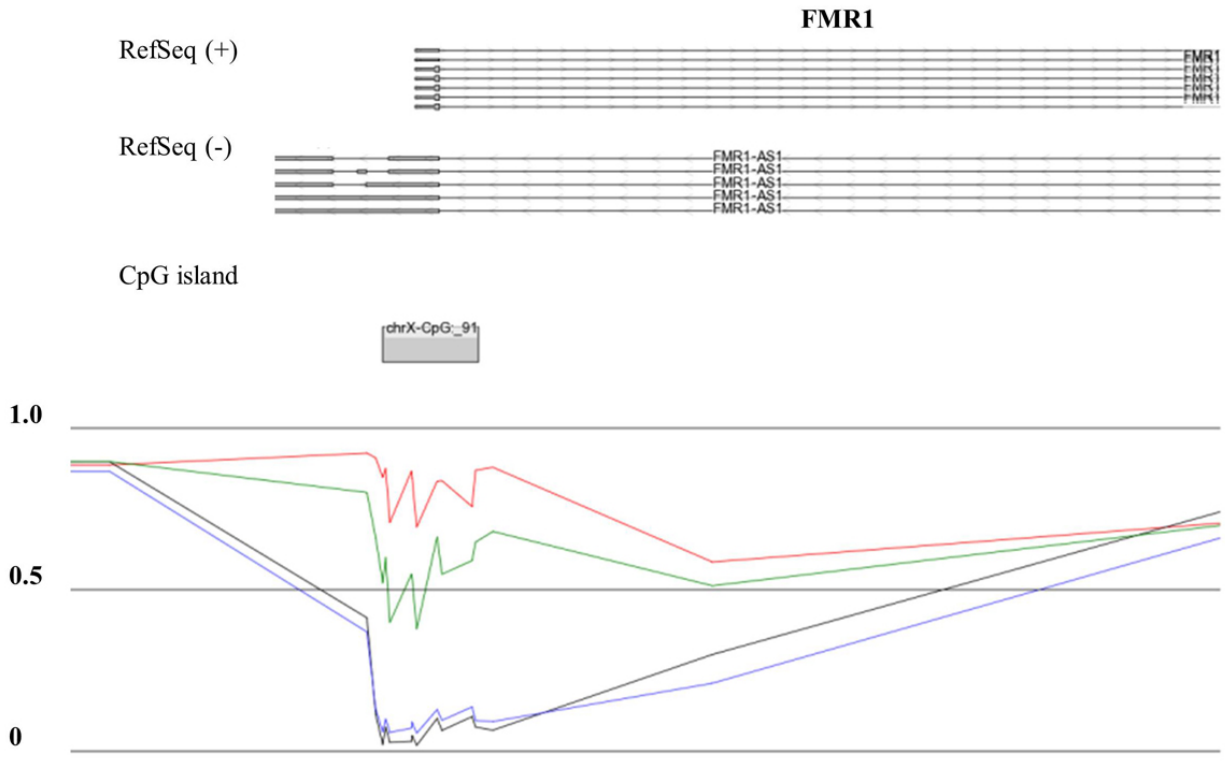

Fig. 1. DNA methylation analysis of FMR1 promoter in FXS male patients versus male controls. The figures show the methylation levels (0 = 0% methylation, 0.5 = 50% methylation, 1= 100% methylation) at specific array probes at the FMR1 gene promoter; the CpG island location; and the Reference sequence (RefSeq) for 5'-3' strand (+) and for 3'-5'strand (-). FMR1 mean methylation levels in males with full mutation (red), Mosaic (green), premutation (blue) and controls (black).

Premutation carriers do not have FXS, but female carriers are at increased risk of having children

with FXS. Current clinical diagnostic tests for FXS are technically challenging and involve the use of complex techniques such as Southern blotting and long range PCR methods. The aim of this study was to clinically validate the use of Illumina Infinium HumanMethylation450 DNA methylation array for FXS screening. We analyzed the DNA methylation in peripheral blood of 32 males previously diagnosed with Fragile X syndrome, including 9 with mosaicism (presence of cells with both normal and full mutation alleles). The study also included five female full mutation carriers, 11 male premutation carriers, and 11 female premutation carriers. The *FMR1* promoter DNA methylation of these subjects was assayed using the array and compared to that of 300 normal controls. Our findings demonstrated the ability of this array to detect all FXS male patients sensitively and to differentiate patients with tissue mosaicisms. Males with full mutation showed robust hypermethylation of the *FMR1* promoter, while males with mosaicism exhibited a reduction of methylation signal (Fig. 1). Female full mutation carriers and individuals with premutation, which typically do not have FXS symptoms, did not show *FMR1* methylation changes. We have clinically validated this genome-wide DNA methylation assay as a sensitive, specific and cost effective alternative for Fragile X syndrome screening.

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Publication

Clinical Validation of Fragile X Syndrome Screening by DNA Methylation Array.
Schenkel LC, Schwartz C, Skinner C, Rodenhiser DI, Ainsworth PJ, Pare G, Sadikovic B
J Mol Diagn. 2016 Nov