

## Abstract 39

### NOVEL EPIGENETIC MARKERS OF THE FRAGILE X ALLELES.

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We have identified a number of novel epigenetic markers for Fragile X Syndrome (FXS), based on the methylation profiles of regions adjacent to the FMR1 CpG island, using MALDI-TOF MS. We named the most promising region Fragile X Related Epigenetic Element 1 (FREE1). Our pilot sample consisted of DNA from 34 healthy controls and 21 FXS individuals, from whole blood, EBV transformed lymphoblasts and Chorionic Villi Samples. FREE1 methylation analysis was used to identify FXS methylated alleles with specificity of >99% and sensitivity greater than 10% methylation. It closely reflected assessment of the FMR1 CpG island using Southern blot analysis, but required ~100 fold less DNA quantity, was more rapid, and ~10 fold less expensive. FREE1 methylation pattern was generally consistent between the cell types and closely reflected adult pattern of X-inactivation. Notably, both the classical CpG island and the FREE1 region escaped methylation related to X-inactivation in 15 weeks' old CVS cells, suggesting that there may be a functional role for both regions in females during late foetal development. To elucidate the relationship between changes in FREE1 methylation and bi-directional transcription at the FMR1 locus, we have treated cell lines with 5-aza-2'-deoxycytidine, and identified critical CpG units within the FREE1 region closely related to FMR1 and FMR4/ASFMR1 expression. These sites were co-localised with putative SRY and GATA1/2 binding regions. In conclusion, we have identified a novel epigenetic marker for FXS – FREE1 using MALDI-TOF MS that may overcome the problems associated with screening for large methylated CGG expansions across different tissues. FREE1 methylation was closely related to that of the FMR1 CpG island, in adult and foetal tissues, and was linked to bi-directional transcription at the locus. Due to the high throughput nature of the assay, it may become an ideal tool for newborn or prenatal screening of FXS.

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