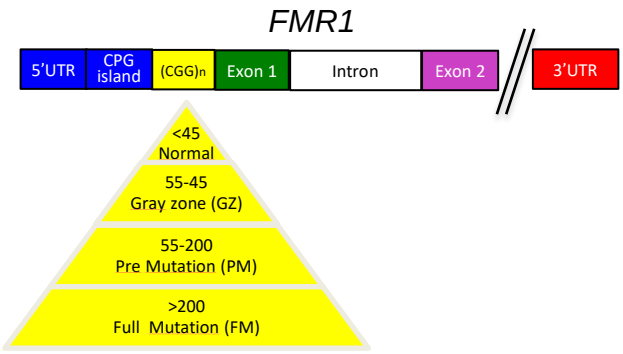
murdoch children's research institute

# FREE epigenetic biomarkers

- Prognostic for Fragile X Syndrome and related disorders
- ASD/developmental delay molecular screening
- Paediatric, newborn and prenatal screening

## The opportunity

Mutations in the *Fragile X mental retardation 1 (FMR1)* gene result in a heterogeneous neurological phenotype, including variable levels of intellectual disability and autistic behaviours. Typically, these mutations comprise trinucleotide expansion of a CGG repetitive sequence.




Gold standard testing for Fragile X Syndrome currently involves Southern blot analysis to determine methylation status of the FMR1 CpG island. However, this method is cumbersome and cannot predict specific cognitive and behavioural impairments in female carriers with mutated *FMR1* alleles.

The alternative PCR-based methods that are presently available lack sensitivity, are not suitable for application in newborn screening or very young children and do not provide prognostic information in females.

Researchers at MCRI have identified novel epigenetic regions located within the *FMR1* gene that overcome the limitations of the current testing regimes.

## The technology

The methylation level of novel epigenetic regions located within the *FMR1* gene, referred to as Fragile X Related Epigenetic Elements, or **FREE biomarkers**, are significantly associated with fragile X mental retardation protein levels, as well as cognitive impairment in males and females with Fragile X Syndrome.

**FREE biomarker** methylation levels have been assessed on more than 6,000 samples and by multiple methods, including matrix-assisted laser desorption/ ionization-time of flight mass spectrometry (MALDI-TOF MS) and PCR-based methods.

**FREE biomarkers provide prognostic information:** methylation ratios are significantly correlated with various measures of intellectual impairment (e.g. verbal IQ impaired full mutation females P=0.002).

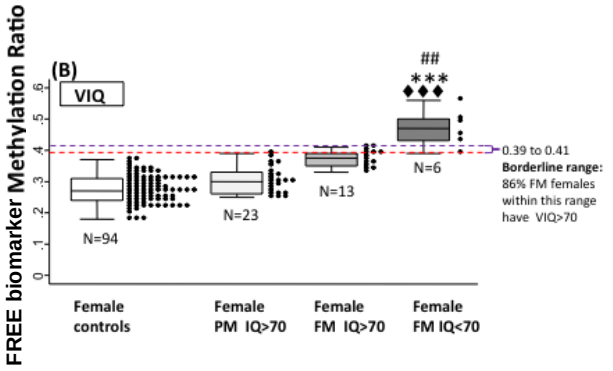



**Figure: 138 females with Verbal IQ scores and CGG expansion size**

This work is referenced in Health Net's recent update of their Genetic Testing Indications National Medical Policy.

**FREE biomarkers are ethically safe for use in newborn screening:** a threshold methylation ratio of 0.39, followed by second line testing involving CGG sizing, could be used in newborn screening to identify all positives carrying a full mutation allele.

This methodology avoids the detection of premutation alleles, which are associated with late onset disorders and would raise the ethical issue of pre-symptomatic testing in children and newborns for currently untreatable and non-preventable disorders with incomplete penetrance.

**FREE biomarkers in ASD/developmental delay molecular screening:** assessment of individuals referred for ASD/ developmental delay will identify those individuals with expansion mosaicism, which return a false negative result using conventional testing methods.

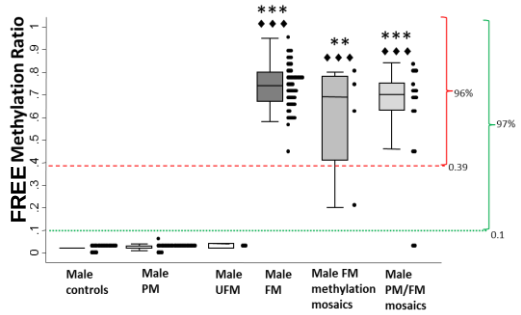



**Figure: 124 males investigated for developmental delay/ASD**

**FREE biomarkers predict dysexecutive-psychiatric phenotype in FMR1 premutation females:** methylation of FREE biomarkers is associated with an increased risk of developing comorbid dysexecutive and social anxiety symptoms in premutation females. These findings could have implications for early intervention and risk estimate recommendations aimed at improving the outcomes for premutation females and their families.

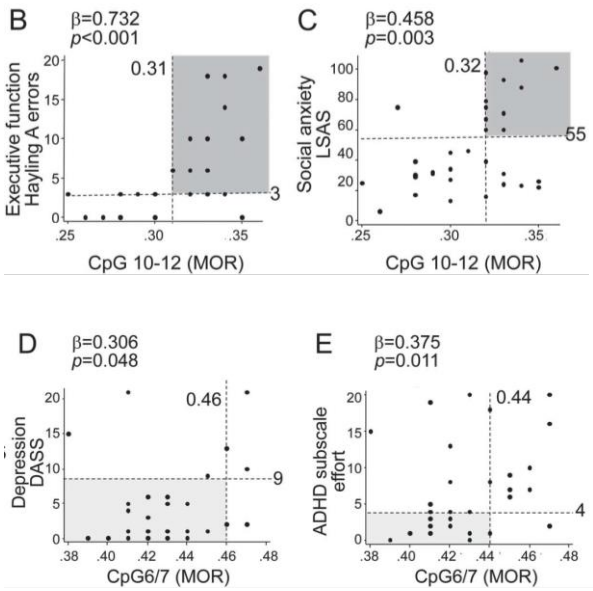



*Figure: Dark gray boxes represent the range for the affected PM group (true positives) based on the applied thresholds. Light gray boxes represent the range for the unaffected PM group (true negatives) based on the applied thresholds. β represents standardized coefficients taking into account relatedness between individuals. MOR = methylation output ratio.*

## Opportunity for partnership

The Murdoch Children's Research Institute is seeking a licensee for this technology.

## Associated technology

Researchers at MCRI have also developed a novel method referred to as "MS-QMA", a high-throughput approach that combines the qualitative nature of high resolution melt analysis and quantitative real-time PCR standard curve method for accurate quantification of methylation in a single assay. MS-QMA has a very low detection limit of ~2% methylation quantification and 1% for qualitative detection.

## Intellectual Property

The Murdoch Children's Research Institute holds a number of patent families relating to numerous **FREE biomarkers**, as well as the MS-QMA method.

Intellectual property, in the form of copyright, also exists in the Q'Max desktop application. The details regarding our 3 Patent Families are:

1)
Patent Family for an assay for determining epigenetic profiles of markers of Fragile X alleles (PCT/AU2010/000169), which is directed towards assessing methylation of FREE1 and FREE2, its status internationally is:
Australia (2010215061): Accepted
Israel (214690): Accepted
Europe (10743317.9): Accepted
USA (13/202,085): Pending

2)
Patent Family for the treatment and diagnosis of epigenetic disorders and conditions (PCT/AU2011/001024), directed towards assessing methylation of FREE2(D) and FREE2(E) regions which have utility for prognosis of late-onset conditions. The status internationally is:
Europe (11815911.0): Pending
US (14/932,634): Pending
Australia (2011288917): Granted

3)
Patent Family for an assay for quantitating the extent of methylation of a target site (PCT/AU2014/000044), this is directed towards the MSQMA assay and the use of this assay in diagnosis:
Europe (14743573.9): Pending
US (14/763,485): Pending
Australia (2014210369): Pending

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## Key publications


Godler DE et. al. Early Detection of Fragile X Syndrome: Applications of a Novel Approach for Improved Quantitative Methylation Analysis in Venous Blood and Newborn Blood Spots. Clinical Chemistry (2014) v. 60, p.963-973.

Aliaga SM et al. 2016 Identification of Males with Cryptic Fragile X Alleles by Methylation-Specific Quantitative Melt Analysis. Clin Chem. February 2016 vol. 62 no. 2 343-352

Inaba Y. et at. 2014 Early detection of fragile X syndrome: applications of a novel approach for improved quantitative methylation analysis in venous blood and newborn blood spots. Clin Chem. 2014 Jul;60(7):963-73. Cornish KM, Kraan CM, et al. (2015) Neurology 84:1–2.

Inaba Y et al. Clin. Chem. (2014) v. 60, p.963-973.

Inaba Y et al. Genet Med. (2013) Apr;15(4):290-8.

Godler DE et al. Clin Chem. (2012) Mar;58(3):590-8.



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