Poster 15. ADVANCES IN FRAGILE-X TESTING

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Introduction: Fragile-X syndrome is the most common form of intellectual disability in the general population, usually caused by an expansion of a trinucleotide CGG repeat in the 5' untranslated region of the *FMR1* gene. In most cases, expansions over 200 repeats, termed full mutations, cause silencing of *FMR1* gene due to methylation of its promoter, and consequently loss of protein product. Expansions with 55-199 CGG repeats called pre-mutations or others even smaller (45-54 CGG repeats) named intermediate, do not cause Fragile-X syndrome, but are frequently associated with late-onset neurological and/or reproductive disorders (FXTAS/FXPOI). Southern Blot is still considered the gold standard for molecular diagnosis of Fragile-X syndrome, because it is able to clearly characterize size and methylation status of full and pre-mutated *FMR1* alleles (following DNA digestion with methylation sensitive enzymes). Nevertheless, Southern Blot is a very time-consuming technique and requires a large amount of intact and high-molecular weight DNA. As such, several methodologies have been developed to replace Southern Blot and overcome its disadvantages.

Purpose and methods: The aim of this work was to test other techniques in order to substitute totally or partially the Southern Blot method, by comparing their capability for *FMR1* allele sizing as well as *FMR1* Methylation status determination. The techniques/kits under investigation are based on PCR, Triplet-Primed PCR and High Resolution Melting Curve Analysis. For this study several DNA samples from patients previously characterized at the molecular level in our laboratory were used.
Although using a very small number of samples, this work describes and compares several different methodologies in an attempt to establish if they can adequately replace Southern Blot.

Results and conclusions: Our results show that the Amplidex® *FMR1* mPCR approach is promising in getting away from Southern Blots applied in Fragile-X testing.

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