## [P-M-138] PROPOSITION FOR A MULTI-STEP MUTATION DETECTION IN HAEMOPHILIA A

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**Introduction:** Affecting one in 10000 male newborns, hemophilia A is the most common hereditary haemorrhagic disorder, caused by a deficiency or dysfunction of blood coagulation Factor VIII (F8). Identification of the causal mutation is important for correct clinical management and early detection of female carriers. The F8 size and its large mutational spectrum represent a challenge for routine genetic testing.
**Methods:** We then developed a rapid multi-step approach (Southern blotting, direct sequencing and MLPA techniques) for the detection of F8 mutations in a large cohort of 98 unrelated haemophilia A patients (60 severe, 38 moderate/mild forms) regularly followed at the Haemophilia Centre of the Cliniques Universitaires Saint-Luc, Brussels.
**Results:** The first step including the detection of the intron 22 and 1 inversion or large deletion/ duplication resulted in the identification of 30 mutations (30/60, 50%) among patients with severe phenotype. The second step based on the relative existence of hot spot exons (europium.csc.mrc.ac. uk) allowed by direct sequencing of exons 7-8, 11-12, 14, 18, 23-24 and 26 identification of 16 additional mutations (6 deletions, 2 insertions, 5 missense, 2 non sense and 1 splice defect) in severe patients (16/60, 27%) and 20 missense mutations in moderate/mild patients (20/38, 53%). In the third step, the remaining exons were analysed leading to the identification of 27 additional sequence changes in 11 severe and 16 moderate/mild patients.
**Conclusions:** This multi-step approach which can be easily implemented in a diagnose laboratory allowed a rapid identification of 95% mutations (93/98) in a cohort of phenotypic heterogeneous families. Interestingly, 19 mutations identified in this study have not been previously reported so far.
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