

## Whole *F8* and *VWF* gene sequencing using Next-Generation Sequencing for mutation-negative French and Canadian hemophilia A patients

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**Background:** The identification of the molecular defect responsible of hemophilia A (HA) is an important component in optimizing disease diagnosis. Despite the analysis of all exons of *F8*, as well as 5'/3' UTR regions and search for copy number variants, the pathogenic variant is still missing in about 5% of HA patients. In the last several years, Next-Generation Sequencing (NGS) has provided the capabilities of sequencing the complete *F8* and *VWF* genes.

**Aims:** The aim of this study is to characterize intronic mutations in the *F8* gene of "mutation-negative" French and Canadian HA patients, by using a whole gene sequencing method performed by NGS.

**Methods:** We developed a small panel containing whole *F8* (195kb) and *VWF* genes (188kb). The SureSelect system was used for target enrichment. We first analyzed 10 mutation-negative HA patients and 6 controls (3 HA and 3 VWD) with known pathogenic variants. NGS was performed on a MiSeq sequencer. Data was analyzed with a bioinformatic pipeline according to the Genome Analysis Toolkit best practices. The effect on splice sites was predicted with Alamut<sup>®</sup> Visual.

**Results:** NGS data revealed 5 deep intronic candidate *F8* variants (one recurrent) in 5 moderate or mild HA patients. Patient 6, who was known as a moderate HA patient, had two exonic variants in *VWF* described as VWD disease-causing. This patient has been reclassified as VWD type 2N/3. Two variants causing gene conversion in *VWF* have been found in patient 7 (mild HA). Some cases with the same variants have been described in VWD patients in France but they were considered as non-pathogenic because always associated with a more pathogenic variant causing the disease. The deleterious effect of this gene conversion between *VWF* and his pseudogene is not well understood. Patient 8 (mild HA) and related patients 9 and 10 (moderate HA) didn't have an identified variant. We also found 5 variants that don't change splice sites so weren't classified as potentially pathogenic. The average sequence coverage depth was 20-50X. Some variants had a coverage <20X threshold and they were all confirmed with Sanger sequencing.

**Conclusion:** This preliminary NGS run has corroborated that HA might be caused by deep intronic variants, by discovering 4 new candidate variants. It also showed the importance of sequencing *F8* and *VWF* at the same time, to identify misdiagnosed patients. Future sequencing will optimize the depth of coverage for this assay. RNA and minigene studies are in progress to confirm the deleterious effect of the intronic variants on *F8* mRNA splicing.