Molecular diagnosis of haemophilia A: four novel variants identified in five patients

Rita Certã¹, Isabel Moreira¹, Ema Antunes¹, Eugénia Cruz¹, Maria João Diniz², Paula Kjollerstrom³, Sara Morais³, João Gonçalves¹

1- Unidade de Genética Molecular, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa. 2- Serviço de Imunohemoterapia, Hospital de S. José, Centro Hospitalar Lisboa Central, Lisboa. 3- Serviço de Hematologia Clínica, Hospital Geral de Santo António, Centro Hospitalar do Porto, Porto. 4- Unidade de Hematologia, Hospital D. Estefânia, Centro Hospitalar Lisboa Central, Lisboa, Portugal.

Introduction:
Haemophilia A (HMA) is an X-linked bleeding disorder caused by reduced levels of the coagulation factor VIII (FVIII) due to alterations in the F8 gene. Decreased levels of FVIII coagulant activity (FVIII:C) leads to a loss of clotting activity and consequent bleeding (predominantly into joints, muscles and inner organs). The severity of HMA ranges from mild (5-30% FVIII:C) to moderate (2-5% FVIII:C) to severe (<1% FVIII:C). During the last five years, we have found four novel variants identified in five index patients with no family history of HMA.

Results:
F8 variant analysis allowed identification of three frameshift and one missense variants: c.1060_1061delCT, c.3561dupT, and c.4804delC detected in families presenting severe HMA; c.5836G>T variant was identified in two unrelated patients with a mild phenotype (Table 1; Figures 1-4). None of these variants had been previously reported.

Methodology:
Analysis of the F8 gene was performed in five index patients (one female from a family without previous molecular studies, index case not available), using genomic DNA extracted from peripheral EDTA blood samples and specific PCR for F8 exons, followed by Sanger sequencing. F8 IVS22 and IVS5 inversions were excluded in severe HMA cases. Bioinformatics analysis was performed with several pathogenicity prediction tools ( Alamut Visual, VarSome, VEP and Human Splicing Finder).

Discussion:
In the three patients with severe HMA, three different novel F8 variants were identified: c.1060_1061delCT, p.(Leu354Thrfs*5) (Fig. 2), c.3561dupT, p.(Pro1188Serfs*10) (Fig. 3) and c.4804delC, p.(Gln1602Lysfs*19). All these variants create a frameshift, leading to a premature termination codon and presumably resulting in non-functional truncated proteins, confirming the patient’s phenotypes.

Idenfication of novel pathogenic F8 variants in HMA patients allows genotype-phenotype correlations, appropriate genetic counseling and new knowledge about the molecular bases of this pathology.

Table 1. Novel F8 variants identified.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Variant type</th>
<th>HMA clinical phenotype</th>
<th>F8 location</th>
<th>Nucleotide change (NM_000132.3)</th>
<th>Amino acid change (NP_000123.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Missense</td>
<td>Mild</td>
<td>Exon 18</td>
<td>c.5836G&gt;T</td>
<td>p.(Asp1946Tyr)</td>
</tr>
<tr>
<td>P2</td>
<td>Frameshift</td>
<td>Severe</td>
<td>Exon 14</td>
<td>c.3561dupT</td>
<td>p.(Pro1188Serfs*10)</td>
</tr>
<tr>
<td>P3</td>
<td>Frameshift</td>
<td>Severe</td>
<td>Exon 14</td>
<td>c.3561dupT</td>
<td>p.(Pro1188Serfs*10)</td>
</tr>
<tr>
<td>P4</td>
<td>Frameshift</td>
<td>Severe</td>
<td>Exon 14</td>
<td>c.4804delC</td>
<td>p.(Gln1602Lysfs*19)</td>
</tr>
</tbody>
</table>

REFERENCES: