Impact of a new genetic variant on FVII: C activity

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Abstract

Background: The prediction of the phenotypic effect of a genetic variant represents a useful tool in genetic counseling. However, in coagulation factor VII (FVII) deficiency there is no straight correlation between genotype and phenotype since the residual FVII coagulant (FVII:C) activity associated with specific genetic variants does not always account for the observed clinical signs.

Objective: to better describe the correlation between genotype and clinical phenotype of F7 gene we report a family case with a deficient FVII:C activity.

Results and discussion: The case under investigation came to our attention during a genetic counseling attended by the proband, because she wanted to know, given the familiarity for breast cancer, her carrier probability for a BRCA1/2 pathogenetic variant. Pedigree analysis showed that besides cancer predisposition both the proband and her two sons suffered from recurrent spontaneous bleeding. Their coagulation pathway analysis was indicative of a FVII:C activity reduction with a pattern mimicking an autosomal dominant inheritance.

Proband F7 sequencing showed the following heterozygous variants: c.1088C>A (p.Pro363His), c.-326_-325insCCTATATCC, c.-122T>C and c.1238G>A. The molecular analysis of her sons highlighted that c.1088C>A variant was in trans configuration. The occurrence of c.1088C>A variant alone was associated with 36% of FVII:C residual activity. Conversely, when this variant was in compound heterozygosity with c.-326_-325insCCTATATCC, c.-122T>C and c.1238G>A haplotype, the FVII:C residual activity further shrunk to 22%.

c.1088C>A variant alone determined the most significant FVII:C activity reduction, however, when found in combination with c.-326_-325insCCTATATCC, c.-122T>C and c.1238G>A haplotype an additive effect on the FVII:C activity phenotype was observed.

Essentials

Atypical clinical phenotypic manifestation in FVII deficiency.

Intra-familial analysis for F7 genetic alterations and related coagulation activity defects.

Combination of two F7 alleles and their additive impact on FVII:C activity reduction.

Importance of intra-familial screening to highlight the novel c.1088C>A F7 genetic variant and its weight on the phenotypic output.

Introduction

Congenital factor VII deficiency is a rare bleeding disorder with an estimated prevalence of 1 person out of 500,000 [1]. The clinical expression of this condition is extremely variable and can be characterized by either asymptomaticity or signs such as epistaxis, gum bleeding, menorrhagia, hematomas and hematuria. The most serious clinical features are due to recurrent hemorrhathosis, cerebral and gastrointestinal hemorrhages and bleeding during surgical procedures.

Prolonged prothrombin time (PT) with a normal activated partial thromboplastin time (aPTT) and FVII coagulant (FVII:C) activity below 70% are considered indicative of this condition [2,3]. The severity of hemorrhagic manifestations is however only partially related to the residual plasmatic FVII:C activity, besides, a wide and non-harmonic operative range of FVII:C activity has been proposed to evaluate the bleeding risk. A FVII:C activity above 8% has been generally associated with a low spontaneous bleeding risk [4]. Values ranging from 15 to 20%, instead, may favor bleeding in case of trauma, surgery and pregnancy [5], whereas an overall FVII:C activity above 20% should guarantee safety even in course of surgery [6].

Individuals carrying an heterozygous variant in the F7 gene are generally asymptomatic as the condition shows an autosomal recessive inheritance pattern. Indeed, only about the 20% of the carriers presents signs such as slight epistaxis, gum bleeding and menorrhagia [7], even though they are not likely to develop a significant clinical symptomatology during their life course.

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In this report, we present a family where the proband and her two sons both show a clinical symptomatology correlated with a low FVII:C activity mimicking an autosomal dominant inheritance pattern. In order to establish the contributory effect of these variants on the activity of the FVII:C we described the correlation between the $F7$ genotypes and both laboratory and clinical investigations.

**Materials and methods**

**Sample collection**

Written informed consent was signed by all screened subjects. Blood samples of the proband and her two sons were collected by standard atraumatic venepuncture technique using 0.1 mol/L trisodium citrate buffer as anticoagulant.

**Coagulation tests**

The FVII:C activity of all family members was determined using a human recombinant and an ox thromboplastin. PT, aPTT and FVII:C activity were measured by a one-stage semi-automated bioassay from plasma specimens in a ST4 coagulometer (Diagnostica Stago, Asnieres, France). Results have been expressed as a percentage of activity of the standard plasma supplied by the manufacturer. According to the manufacturer the normal ranges of PT and aPTT were 70-100% and 30-40 sec, whereas, those of FVII:C were 80.0-120.0% for both the human recombinant and the ox thromboplastin test, and 70.0 – 120.0% for the coagulometric test.

**DNA analyses**

Genomic DNA of all patients was extracted from whole blood using Qiagen DNA extraction Kit (QIAGEN, Dusseldorf, Germany). Next-Generation (NGS)/Massively Parallel Sequencing (MPS) of the coding regions of $F7$ gene was performed using an Illumina Custom Panel (Illumina, San Diego, CA 92122). Either large deletions or duplications in $F7$ gene were directly sequenced as previously described [8]. Both gene were directly sequenced as previously described [8]. Both gene were directly sequenced as previously described [8]. Both gene were directly sequenced as previously described [8]. Both gene were directly sequenced as previously described [8]. Both gene were directly sequenced as previously described [8]. Both gene were directly sequenced as previously described [8].

**Results and discussion**

Besides breast cancer predisposition, the proband had previously complained epistaxis, menorrhagia, gum bleeding and spontaneous hematomas, as well as her two sons, who both suffered from recurrent spontaneous bleeding.

**Table 1. Genotype and laboratory features revealed in the family members**

<table>
<thead>
<tr>
<th>Allelic 1 Variant</th>
<th>Allelic 2 Variants</th>
<th>PT %</th>
<th>aPTT sec</th>
<th>FVII:C %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>human thromboplastin</td>
<td>ox brain thromboplastin</td>
<td>coagulometric method</td>
</tr>
<tr>
<td>Proband</td>
<td></td>
<td>c.1088C&gt;A</td>
<td>c.-122T&gt;C; c.326,-325insCCTATATCC; 1238A&gt;G</td>
<td>56.9 (70.0-100.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.122T&gt;C</td>
<td>c.326,-325insCCTATATCC; 1238A&gt;G</td>
<td>33.7 (80.0-120.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.326,-325insCCTATATCC; 1238A&gt;G</td>
<td>88.7 (80.0-120.0)</td>
<td>63.5 (80.0-120.0)</td>
</tr>
<tr>
<td>First born</td>
<td></td>
<td>c.1088C&gt;A</td>
<td>c.122T&gt;C</td>
<td>94.0 (70.0-100.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.326,-325insCCTATATCC; 1238A&gt;G</td>
<td>47.9 (80.0-120.0)</td>
<td>20.6 (80.0-120.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.122T&gt;C</td>
<td>67.7 (70.0-100.0)</td>
<td>28.9 (30.0-40.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.122T&gt;C</td>
<td>67.7 (70.0-100.0)</td>
<td>28.9 (30.0-40.0)</td>
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<tr>
<td></td>
<td></td>
<td>c.122T&gt;C</td>
<td>67.7 (70.0-100.0)</td>
<td>28.9 (30.0-40.0)</td>
</tr>
</tbody>
</table>

PT: prolonged prothrombin time; aPTT: activated partial thromboplastin time; FVII:C: FVII coagulation activity.
Specifically, it leads to the replacement of Proline 363 (CCC) by a Histidine (CAC) residue in the protease domain of F7 gene and it is predicted to be harmful by the main in silico tools. Proline and Histidine may be considered to have only moderate physicochemical differences (Grantham dist.: 77 (0-215)) however, it is worth to consider that Proline has an exceptional constrained conformation linked to its alpha-N-rigid-ring structure, and its presence in the protein chain may strongly affect the protein secondary structure. As a consequence, the substitution of the Proline with a less constrained Histidine residue could increase the grade of freedom of the protein chain influencing in this way the FVII protease activity.

The c.1238G>A variant leads to the change of an Arginine with a Glycine residue in position 413 (p.Arg413Gln) and is predicted to be benign/neutral by the main in silico tools. Previous studies indicate that such genetic variant, when in an heterozygous state accounts for about 25-30% decrease of plasma FVII:C activity [10-15]. However, its net contribution is still unclear since it is in linkage disequilibrium with the c.-326_-325insCCTATATCC. The genotypic effect on FVII:C activity may be further intricated by the complete association of c.-122T>C with the c.-326_-325insCCTATATCC variant, which might account for the strong reduction of the promotor activity of the F7 gene [16].

The intra-familial comparison between the phenotype of the proband and the one of her two sons allowed us to establish that the c.1088C>A variant, in heterozygous state, is the most important genetic feature for the reduction of the FVII:C activity, being it associated with 36% of residual activity evaluated by coagulometric assay. Indeed, said residual activity further decreases to 22% when this genetic variant is in compound heterozygosity with the c.-326_-325insCCTATATCC, c.-122T>C and c.1238G>A haplotype.

Therefore, the variant c.1088C>A showed a different expression compared with the c.-326_-325insCCTATATCC, c.-122T>C and the c.1238G>A haplotype, mainly contributing into FVII:C activity reduction. However, the observed decrease of FVII:C activity indicates that the combination of two F7 gene alleles provides an additive effect on the dampening of FVII:C activity, even if of no relevant clinical significance [17,18]. Interestingly, the net effect on the FVII:C activity drop due to the c.-326_-325insCCTATATCC, c.-122T>C and the c.1238G>A haplotype was greater than expected (39% vs 25-30%), making difficult the prediction of their combined effect.

Despite its inter-individual variability, the might of this study is represented by the simultaneous analysis of all the family members performed by homogeneous analytical protocols in order to achieve a better establishment of the variant contributory effects on FVII:C activity. In conclusion, we report for the first time the combination of the c.1088C>A variant with the most common c.-326_-325insCCTATATCC, c.-122T>C, c.1238G>A haplotype and describe their, never analyzed before, impact on FVII:C activity through an additive model that may contribute to better classify the observed clinical phenotype.

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Disclosure of conflicts of interest
The authors declare no conflicts of interest.

Author contributions
Study concept and design: G. Miolo; sample processing and measurements: G. Tessitori and A. Percesepe; processing and interpretation of data: G. Miolo, G. Tessitori and A. Percesepe; drafting of the manuscript: G. Miolo; critical revision of the manuscript: L. Caggiari, M. De Zorzi, M. Tedeschi, D. A. Santeufemia, A. Steffan, V. De Re and G. Corona.

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