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 shrunked 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 hemorrhaxis, 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 heterogeneous 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 (MPSS) 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 investigated by Multiplex Ligase-Dependent Probe Amplification scan (MLPA) (SALSA P207-F9 probeeks–MRC-Holland). The variants placed in the 5’ untranslated region (5’UTR) of the F7 gene were directly sequenced as previously described [8]. Both sons were screened only for the variants identified in the proband.

**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 |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | PT %                        | aPTT sec                    | Allelic 1 Variant            | Allelic 2 Variants           | FVII:C %                     |
|                            | human thromboplastin        | ox brain thromboplastin     | coagulometric test           |                             |                             |
| Proband                    | 56.9 (70.0-100.0)           | 30.7 (30.0-40.0)            | c.1088C>A                    | c.1227C; c.326,-325insCCTATATCC; c.1227C>G; c.1238G>A | 33.7 (80.0-120.0)            |
|                            | (70.0-100.0)                | (30.0-40.0)                 |                             |                             | 11.9 (80.0-120.0)            | 22 (70.0-120.0)             |
| First born                 | 94.0 (70.0-100.0)           | 35.4 (30.0-40.0)            | c.1088C>A                    | c.1227C; c.326,-325insCCTATATCC; c.1238G>A | 88.7 (80.0-120.0)            |
|                            | (70.0-100.0)                | (30.0-40.0)                 |                             |                             | 63.5 (80.0-120.0)            | 61 (70.0-120.0)             |
| Second born                | 67.7 (70.0-100.0)           | 28.9 (30.0-40.0)            | c.1088C>A                    |                             | 47.9 (80.0-120.0)            |
|                            | (70.0-100.0)                | (30.0-40.0)                 |                             |                             | 20.6 (80.0-120.0)            | 36 (70.0-120.0)             |

PT: prolonged prothrombin time; aPTT: activated partial thromboplastin time; FVII:C: FVII coagulation activity.
The authors declare no conflicts of interest.

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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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