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Letter to the Editors-in-Chief

A novel allele variant of the SERPINF2 gene responsible for severe plasmin inhibitor (α_2-antiplasmin) deficiency in an Italian patient

(α_2-)plasmin inhibitor (PI, also called α_2-antiplasmin), the glycoprotein coded by the SERPINF2 gene, is the major physiological plasmin inhibitor and together with plasminogen activator inhibitor 1 and thrombin activatable fibrinolysis inhibitor plays a major role in regulating fibrinolysis [1,2]. A mild to moderate bleeding diathesis has been reported in subjects heterozygous for PI deficiency, while severe PI deficiency is associated with spontaneous and, more frequently, trauma-induced, severe bleeding resembling hemophilia and factor XIII deficiency. Main symptoms are umbilical stump bleeding, cerebral bleeding, epistaxis, hemarthrosis, menorrhagia, and excess bleeding after surgery or minor trauma. Spontaneous intramedullary hematoma in tubular bones has been reported in 4 homozygotes from 2 families [3].

Given its rarity, the prevalence of homozygous/compound heterozygous PI deficiency is unknown.

1. Case report

Eight hours after dental implant surgery, a 62-year-old man suffered bleeding in the floor of the mouth, with the hematoma extending to the subcutaneous tissue of anterior neck and chest (Fig. 1a). At the age of 15 he had suffered delayed bleeding after tooth extraction. Military service as parachutist was uneventful. At the age of 35 he bled severely after surgery for mastoiditis, requiring surgical revision and red blood cells transfusions. Six years later he was again submitted to surgery for recurrence of mastoiditis without bleeding complications. Dental extractions later were uneventful, but frequent episodes of prolonged epistaxis occurred before hospitalization at the age of 51 because of an atraumatic cerebral temporal hematoma, attributed to untreated hypertension. On that occasion the patient developed, after an intramuscular injection, a large hematoma of the gluteus (Fig. 1b). Platelet counts, routine coagulation tests, bleeding time, and FXIII were within normal limits.

The patient denies consanguinity of his parents. Family history (parents, 2 brothers, 3 sisters and one son) is negative for bleeding, but for a daughter who died of cerebral hemorrhage in a car accident at the age of 15 years. Currently, the patient is suffering of pulmonary emphysema, but is physically active (bicycling) and his liver and kidney function parameters are within normal limits.

Venous blood samples from the propositus and two of his relatives (one sister and son) were collected in vacutainer tubes containing 0.109 M sodium citrate (blood to anticoagulant ratio 9:1) and either tested within 30 min (PFA-100), or centrifuged at 2000*g* for 10 min at room temperature. Platelet poor plasma was transferred into Nalgene tubes and aliquots (0.5 ml) were stored at −80 °C until testing. Instrumentation (STA-R) and reagents for clotting tests (prothrombin time, PT, activated partial thromboplastin time, aPTT, thrombin time, TT, thrombin coagulase time, TCT, fibrinogen, D-dimer), von Willebrand factor antigen, and plasminogen were from Stago (Asnieres sur Sein, France). Factor XIII was measured by an enzymatic method (Behrichrom® FXIII, Siemens Healthcare Diagnostics Products, Marburg, Germany). Platelet function was investigated by Platelet Function Analyzer (PFA)-100 using epinephrine-collagen and adenosine-diphosphate-collagen cartridges (Siemens). PI amidolytic activity was measured by using an ELISA reader (Multiskan-GO, Thermo Scientific, Vantaa, Finland) and by reproducing the conditions of the immediate plasmin inhibition test [4] with Stago reagents in 96 well-ELISA plates (Costar 3590, Corning, NY, USA). Briefly, 50 µl of plasmin diluted in Owren buffer to obtain a final assay concentration of 0.26 nKat/ml were added to a premixture of platelet poor plasma diluted in Owren buffer containing 0.12 mol/l monomethylamine-HCl (final assay concentration 1.1%, 50 µl), and chromogenic substrate CBS 10.85 (50 µl, final assay concentration 0.66 µmol/ml,), followed by immediate absorbance recording at 37 °C (405 nm). Results are average of quadruplicate measurements. PI antigen levels were measured by a commercially available sandwich ELISA using a monoclonal capture antibody (mouse anti-human Serpin F2, epitope unknown) and a biotinylated polyclonal goat anti-human SerpinF2 antibody (DuoSet ELISA, R&D Systems, Minneapolis, MN, USA).

High-throughput DNA sequence analysis was used for mutation screening of the PI gene (SERPINF2) on leukocyte DNA from peripheral citrated blood samples, according to the procedure previously described [5]. Briefly, all the exons, introns and 5'UTR of the gene were amplified using forward and reverse PCR primer designed according to the DNA sequence reported in the literature (reference sequence NG_013215). PCR primers were designed to amplify and sequence < 500 bp amplicons, and overlapping PCR amplicons were designed for all exons > 400 bp to ensure complete coverage. For each PCR reaction, 50 ng of genomic DNA was used. All the PCR product was sequenced on ABI PRISM 3130 Genetic Analyzer sequencer (PE Biosystems, Foster City, CA, USA). All the frameshift and non-sense mutations were scored as pathological mutations. Point mutations were excluded if they were synonymous or included in SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP).

Laboratory parameters of the propositus and his two relatives are reported in Table 1. Coagulation and platelet-related parameters were within normal limits. PI activity levels, as measured by reproducing the conditions of the immediate plasmin inhibition test, and PI antigen levels were < 1% in the propositus, 45% and 40% of normal in his son, and normal in the propositus' sister.

Direct DNA sequencing of all 10 exons identified a nonsense mutation in exon 6, where thymine replaced cytosine at nucleotide 558, resulting in the substitution of Arg with a stop codon at residue 161 (Uniprot numbering, Supplementary Fig. 1). The propositus was homozygous for the substitution. This genetic defect has not been previously reported and was not found in 100 unrelated healthy

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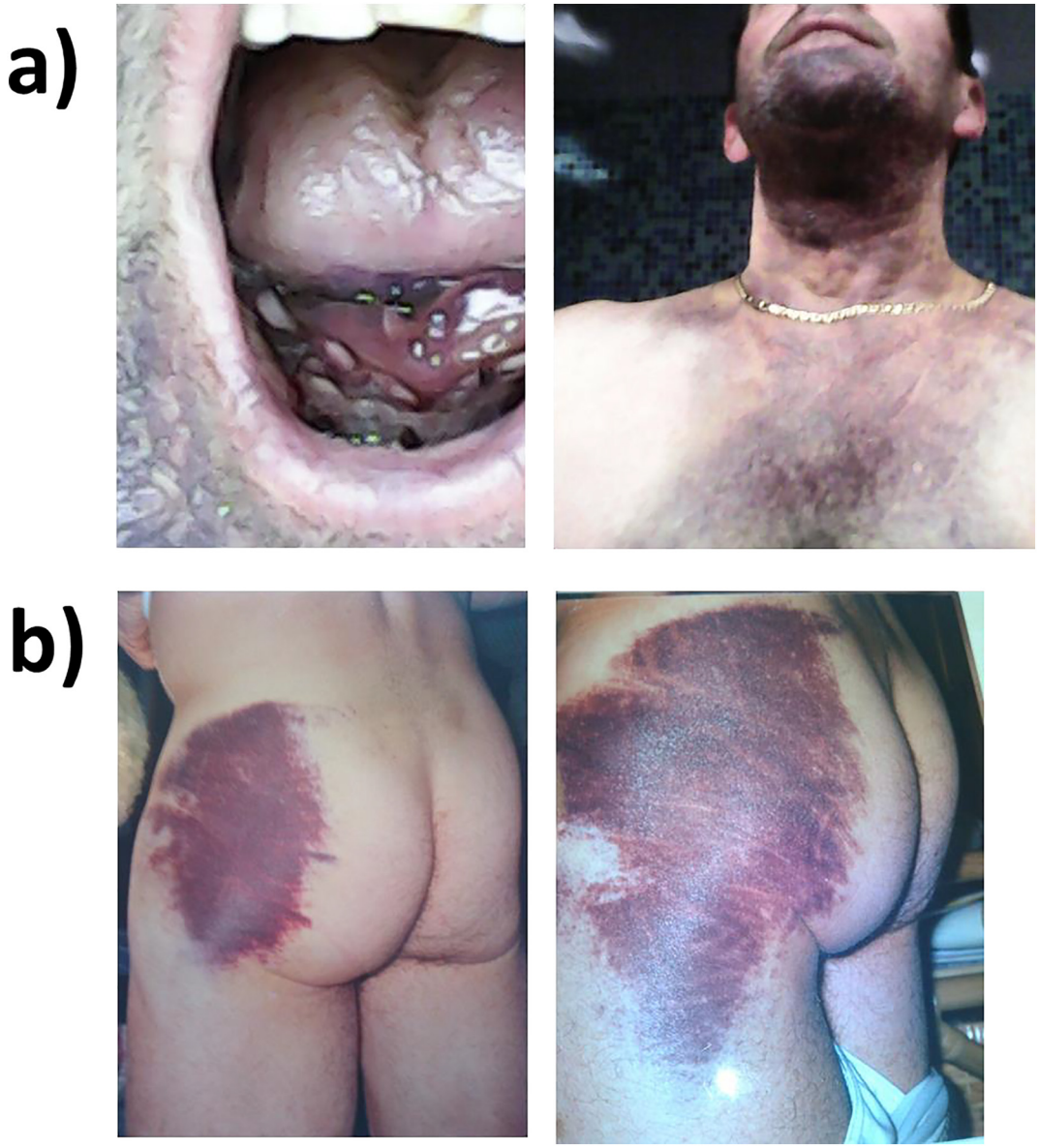

Fig. 1. Bleeding manifestations of the propositus. Panel a): hematoma of the floor of the mouth extending to the subcutaneous tissue of neck and chest after dental implant surgery. Panel b): hematoma of the gluteus after an intramuscular injection occurring during a previous hospitalization for intracerebral hemorrhage.

Table 1
Hemostasis and fibrinolysis parameters of the propositus and two relatives.

	Propositus	Sister	Son	Reference values
PT ratio	1.00	nd[a]	nd	0.90–1.18
aPTT ratio	0.88	nd	nd	0.75–1.29
TT ratio	0.91	nd	nd	0.81–1.25
TCT ratio	0.91	nd	nd	0.81–1.25
Fibrinogen, mg/dl	240	nd	nd	150–400
D-dimer (mg/l)	0.44	nd	nd	0.26–0.77
Platelet count ($\times 10^9$/l)	247	nd	nd	130–400
PFA-100 EPI, sec	88	nd	nd	62–164
PFA-100 ADP, sec	92	nd	nd	64–129
VWF:Ag, %	168	nd	nd	47–138
FXIII activity %	114	135	100	52–156
α_2-Plasmin inhibitor activity, %	< 1.0	108	45	65–140
α_2-Plasmin inhibitor antigen, %	< 0.1	120	40	nd

[a] Not determined.

subjects used as controls. His son was heterozygous for this mutation, whereas his sister had two normal alleles.

2. Discussion

The human SerpinF2 gene is located on chromosome 17 [6] and contains 10 exons and 9 introns spanning about 16 kilobases of DNA [7]. The molecular abnormality responsible for PI deficiency has been characterized in patients from 8 families. A trinucleotide (GAA) deletion in exon 7 leading to absence of glutamine at residue 137 of the mature protein and a frameshift mutation due to a cytosine insertion in exon 10 leading to an elongated protein with 166 additional amino acids are the abnormalities of the Okinawa [8] and Nara [9] quantitative PI deficiencies. In both cases, the misfolded inhibitor protein is degraded within the cell by proteasomes instead of being secreted by the Golgi apparatus [10,11]. Frameshift mutations in exons 5 and 10, due to a thymine deletion at nucleotide 332 [12] and a cytosine deletion at nucleotide 11,309 [13], result in truncated proteins of 94 and 346 AA respectively, with the shorter protein shown to undergo

intracellular degradation, and the longer protein associated with undetectable PI antigen levels. A guanine to adenine mutation in a splicing donor site of intron 2 also results in a truncated protein of 25 AA resulting undetectable in media and lysates of cells transfected with the mutant PI expression vector [14]. In heterozygous patients, type I PI deficiency was found in association with a guanine to adenine mutation in exon 10, converting Val 384 to Met [15], and with the same transition in a splicing donor site of intron 6, leading to exon 6 skipping [16]. The molecular abnormality in the single qualitative PI deficiency reported in the literature is a trinucleotide insertion in exon 10, responsible for an additional Ala in position 356, which transforms the inhibitor into a plasmin substrate [17].

In association with undetectable antigen levels, we report a novel point mutation in exon 6 resulting in the substitution of Arg 161 with a premature termination codon and an expected truncated protein of 160 AA, inclusive of the 27 residues signal peptide. The epitope recognized by the monoclonal antibody used to measure PI antigen is unknown, and we cannot rule out the possibility of a type II deficiency in our patient. Alternatively, intracellular degradation and/or nonsense-mediated mRNA decay, an intron-dependent mechanism which eliminates messenger RNA bearing a premature termination codon [18], may explain the absence of circulating PI in our homozygous patient. To our knowledge, this is the first Italian patient with an established diagnosis of severe PI deficiency; his bleeding history is consistent with published reports [3,19], describing moderate to severe bleeding episodes both spontaneous and/or post-trauma or surgery.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2018.04.006.

Disclosures

The Authors have no conflict of interest to disclose.

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