Contents lists available at ScienceDirect

Acta Haematologica Polonica

journal homepage: www.elsevier.com/locate/achaem

Case report/Kazuistyka

Antithrombin Rouen IV mutation in Polish patient with deep vein thrombosis

Kamil Brutkowski [1], *Ewa Wypasek* [1,2], *Javier Corral* [3], *Anetta Undas* [1,2,*]

[1] *John Paul II Hospital, Cracow, Poland*
[2] *Institute of Cardiology, Jagiellonian University School of Medicine, Cracow, Poland*
[3] *Centro Regional de Hemodonación, Universidad de Murcia, Murcia, Spain*

ARTICLE INFO

Article history:
Received: 07.07.2015
Accepted: 15.10.2015
Available online: 26.10.2015

Keywords:
- Antithrombin deficiency
- SERPINC1 mutation
- Rouen IV
- Venous thromboembolism

ABSTRACT

Antithrombin (AT) deficiency is a rare autosomal dominant disorder which increases the risk of venous thromboembolism (VTE). We report here the case of type II antithrombin deficiency in 44-year-old man who developed left leg deep vein thrombosis (DVT). All exons of *SERPINC1*, the gene encoding AT were amplified by PCR followed by direct sequencing. A heterozygous mutation c.166C>T in exon 2 causing the amino acid substitution of Arg to Cys at residue 56, was found. This mutation does not affect the folding and secretion of the protein, but impairs the heparin affinity, reducing the anticoagulant activity of AT.

To the best of our knowledge, this is a first report of AT type IIHBS deficiency related to Polish patient who experienced DVT.

Introduction

Antithrombin (AT) is a 58 kDa glycoprotein synthesized in the liver, which belongs to the serine protease inhibitors superfamily (serpins) and play a key role as a natural blood anticoagulant. AT forms inactive complex with thrombin and activated factor Xa that prevents blood clotting. Presence of heparin increases this activity 1000-fold [1].

The human gene encoding AT (*SERPINC1*) is located on the chromosome 1q23-25 and consists of 7 exons and 6 introns [1, 2]. Currently, over 300 mutations causing AT deficiency have been reported [3]. More than half of these genetic disorders are missense mutations, which lead to dysfunctional proteins. The others like insertions, deletions and nonsense mutations, typically cause disruption or absence of gene product [4]. Hitherto, there were identified 14 different mutations corresponding to AT deficiency among the Polish patients [5].

Inherited AT deficiency is an autosomal dominant disorder that concerns between 1:600 and 1:5000 of total population [6]. There are two types of inherited AT deficiency, both are associated with reduced AT activity but only type I is related to lower AT antigen levels in blood. Type II can be

* *Corresponding author at*: Instytut Kardiologii, Uniwersytet Jagielloński Collegium Medicum, ul. Prądnicka 80, 31-202 Kraków, Poland. Tel.: +48 12 614 3004; fax: +48 12 423 3900.
E-mail address: mmundas@cyf-kr.edu.pl (A. Undas).
http://dx.doi.org/10.1016/j.achaem.2015.10.001
0001-5814/

classified into three groups depending on the location of the mutations: type IIRS is characterized by reactive site defects, type IIHBS is associated with heparin-binding site and type IIPE is caused by mutations clustered in the C-terminus region of AT protein [7]. AT deficiency increases 20-fold the risk of venous thromboembolism (VTE) [8].

We report here the first Polish case of AT type IIHBS deficiency caused by heterozygous point mutation c.166C>T, p.Arg56Cys (Human Genome Variation Society numbering system) in second exon of *SERPINC1* gene.

Case report

A 45-year-old overweight male patient 5 months after idiopathic left-sided deep vein thrombosis (DVT) was referred for thrombophilia screening. Six months before the incident he suffered a left tibia injury but without the need of plaster. Venous duplex ultrasound scans showed popliteal and superficial femoral veins reflux and thrombi within the tibial veins.

After a trauma the patient was treated with rivaroxaban (Xarelto) 20 mg/d and this therapy is still continued. No signs of bleeding or DVT recurrence were observed during 12 months of therapy.

The proband was the only child. Family history revealed lower limb varices without documented VTE in his mother. The proband's father died of myocardial infarction (MI) before the age of 60. A 16-year proband's son remained asymptomatic.

The most common thrombophilic factors, including Factor V Leiden and prothrombin G20210A mutations, were absent. Plasma protein C, free protein S, antiphospholipid antibodies, factor FVIII and homocysteine were within normal limits. Lupus anticoagulant was negative.

Antithrombin anti-FX activity, measured by two different chromogenic assays: (Berichrom AT, Siemens, Marburg, Germany, reference range 75–125%, and HemosIL, Instrumentation Laboratory SpA, Milano, Italy, reference range 80–120%), were 56% and 49%, respectively. AT antigen levels were performed using a home-made sandwich ELISA kit (reference range 80–120%) and was 98%.

Moreover, plasma AT was evaluated by electrophoretic analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) performed in 8% polyacryamide gel and native PAGE with the presence and absence of 6 M urea as described previously [9]. After separation, proteins were transblotted onto polyvinylidene fluoride (PVDF) membrane. AT was immunostained with rabbit anti-human AT polyclonal antibody (Sigma, Madrid, Spain) and followed by donkey anti-rabbit IgG horseradish peroxidase (HRP) conjugate (GE Healthcare, Barcelona, Spain) with detection by ECL kit (Amersham Bioscences, Piscataway, NJ). Analyses did not identify any abnormal bands that might correspond to variant AT, except native gels, which shows two bands, one with faster electrophoretic mobility and similar intensity to the wild type, which is typical of mutations affecting arginine residues at the heparin binding domain.

After obtained informed written consent, the blood sample was collected in EDTA for DNA analysis. The 7 exons and flanking regions of *SERPINC1* gene were amplified using polymerase chain reaction (PCR) followed by direct DNA sequencing. A heterozygous point mutation c.166C>T that causes a missense protein sequence change p.Arg56Cys (number considering the whole protein) in exon 2 was found.

In addition, protein in silico analysis using PoliPhen-2 and Sorting Intolerant From Tolerant (SIFT) were performed. The obtained result classifies this mutation as 'probably damaging' with score 0.999.

Discussion

To the best of our knowledge, this is a first report of AT type II HBS deficiency detected in a Polish patient who experienced DVT.

The mutation identified in this patient was previously described by Perry and Carrell in 1989 in a 25-year-old man who developed an unexpected coronary thrombosis. This report demonstrated that the described mutation caused a variant known as 'Antithrombin Rouen IV' [10]. It has been also reported by Luxembourg et al. that 1 out of 272 patients with reduced values of AT activity/AT antigen and thromboembolic event, and with positive family history for DVT, carried the p.Arg56Cys variant [11]. However, a recent cohort study shows that the carriers of IIHBS AT deficiency have a 80% lower risk of VTE and 6-fold higher risk of arterial thromboembolism than carriers of other type of AT deficiency [4]. These mutations are usually combined with other thrombotic risk factors in patients with VTE [12]. We show here uncommon clinical presentation of Antithrombin Rouen IV since our patient developed DVT without additional risk factors.

Antithrombin Rouen IV occurs at CpG dinucleotides (CpGs) which are the hotspots for point mutations in *SERPINC1* gene [10]. The cytosine (C) residue in the CpG region is methylated and the product of this process (5-methylocytosine) may be spontaneously deaminated forming thymine (T). This change is not enzymatic repaired and results in a C to T transition on one DNA strand and consequently, change G to A on a opposite DNA strand.

It has been shown that the CpGs mutations are mainly involved in the development of AT type IIHBS deficiency in opposition to type IIRS and IIPE [10, 13].

Arginine at residue 56 is located in N-terminal sequence and is involved in protein conformational changes induced by binding of heparin [11]. Replacement of this amino acid by cysteine has the influence on decreased heparin affinity. It is important to point out that certain functional methods for measuring AT activity in the presence of heparin fail to detect this mutation [14].

In conclusion, our report supports the need for thrombophilia screening in VTE patients below 50 years of age, especially in the absence of the established risk factors. Similar strategy should be considered in arterial thrombosis at young age as evidenced for instance in our report on protein C deficiency detected after MI in a young man [15]. Appropriate and timely anticoagulation in symptomatic patients with inherited AT deficiency can reduce the risk of recurrent thrombotic events. Life-long anticoagulant treat-

ment should be considered in AT deficient patients if they experienced a first-ever idiopathic VTE episode as well as those with two episodes. Prophylactic anticoagulation in asymptomatic carriers, especially if family history for VTE is present may be used in certain high-risk state such as pregnancy or surgery.

Authors' contributions/Wkład autorów

EW – study design, data collection and interpretation, manuscript preparation, literature search. AU – study design, data collection and interpretation, funds collection. JC – data collection and interpretation, literature search. KB – data collection and interpretation, manuscript preparation, literature search.

Conflict of interest/Konflikt interesu

None declared.

Financial support/Finansowanie

This research was supported by the Polish National Science Center (NCN) Grant No. UMO-2013/09/B/NZ5/00254.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

REFERENCES/PIŚMIENNICTWO

[1] Kottke-Marchant K, Duncan A. Antithrombin deficiency: issues in laboratory diagnosis. Arch Pathol Lab Med 2002;126:1326–1336.
[2] Patnaik MM, Moll S. Inherited antithrombin deficiency: a review. Haemophilia 2008;14:1229–1239.
[3] Human Gene Mutation Database; 2015, http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SERPINC1.
[4] Luxembourg B, Pavlova A, Geisen C, Spannagl M, Bergmann F, Krause M, et al. Impact of the type of SERPINC1 mutation and subtype of antithrombin deficiency on the thrombotic phenotype in hereditary antithrombin deficiency. Thromb Haemost 2014;111:249–257.
[5] Szymańska M, Wypasek E, Undas A. Niedobór antytrombiny – problemy diagnostyki laboratoryjnej. Diagnostyka Laboratoryjna 2013;49:145–152.
[6] Cooper PC, Coath F, Daly ME, Makris M. The phenotypic and genetic assessment of antithrombin deficiency. Int J Lab Hematol 2011;33:227–237.
[7] Picard V, Chen JM, Tardy B, Aillaud MF, Boiteux-Vergnes C, Dreyfus M. Detection and characterization of large SERPINC1 deletions in type I inherited antithrombin deficiency. Hum Genet 2010;127:45–53.
[8] Van Boven HH, Vandenbroucke JP, Briet E, Rosendaal FR. Gene–gene and gene–environment interaction determines risk of thrombosis in families with inherited antithrombin deficiency. Blood 1999;94:2590–2594.
[9] Corral J, Huntington JA, González-Conejero R, Mushunje A, Navarro M, Marco P. Mutations in the shutter region of antithrombin result in formation of disulfide-linked dimers and severe venous thrombosis. J Thromb Haemost 2004;2:931–939.
[10] Perry DJ, Carrell RW. CpG dinucleotides are 'hotspots' for mutation in antithrombin III gene. Twelve variants identified using the polymerase chain reaction. Mol Biol Med 1989;6:239–243.
[11] Luxembourg B, Delev D, Geisen C, Spannagl M, Krause M, Miesbach W. Molecular basis of antithrombin deficiency. Thromb Haemost 2011;105:635–646.
[12] Martínez-Martínez I, Navarro-Fernández J, Østergaard A, Gutiérrez-Gallego R, Padilla J, Bohdan N, et al. Amelioration of the severity of heparin-binding antithrombin mutations by posttranslational mosaicism. Blood 2012;120:900–904.
[13] Bayton T, Lane D. Antithrombin Mutation Database. Department of Hematology Imperial College of Medicine; 2015 http://www1.imperial.ac.uk/departmentofmedicine/divisions/experimentalmedicine/haematology/coag/antithrombin/.
[14] Corral J, Vicente V. Puzzling questions on antithrombin: diagnostic limitations and real incidence in venous and arterial thrombosis. Thromb Res 2015;135:1047–1048.
[15] Wypasek E, Pankiw-Bembenek O, Potaczek DP, Alhenc-Gelas M, Trebacz J, Undas A. A missense mutation G109R in the PROC gene associated with type I protein C deficiency in a young Polish man with acute myocardial infarction. Int J Cardiol 2013;167:e146–e148.