RESEARCH LETTER

# New *SERPINC1* gene mutations in patients with antithrombin deficiency: antithrombin Lodz I, II, III, and IV

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**Introduction** Antithrombin (AT) deficiency results in excessive thrombin generation and increased risk of venous thromboembolism (VTE), with congenital deficit being an established risk factor. Inherited AT deficiencies are rare, affecting about 1 in 5000 people, in whom the risk of VTE rises 20- to 40-fold[1] as compared with the general population. In patients with VTE, AT deficiency occurred with a prevalence between 1 in 20 and 1 in 200 cases.[2] The gene encoding AT (*SERPINC1*) is located on chromosome 1q25.1 and comprises 7 exons and 6 introns. Over 90% of mutations detected in *SERPINC1* that lead to AT deficiency are caused by point mutations.[3] The first Polish patient with AT deficiency was reported in 2011 (AT Krakow).[4] Subsequently, genetic analyses of 18 Polish families[5] and 35 patients from the southern region of Poland[6] were published. The current report presents a series of patients suspected of inherited AT deficiency from the Łódź region in central Poland.

**Patients and methods** In 15 patients with AT deficiency and a personal (n = 14) or family (n = 8) history of VTE, genetic analysis of *SERPINC1* was performed. All patients signed an informed consent form. The diagnostic criteria of VTE and stroke were as previously described.[6]

The samples for DNA isolation were collected in EDTA tubes, aliquoted, and stored at –80 °C until analysis. Genetic analysis of *SERPINC1* was performed using nanopore sequencing of long-range polymerase chain reaction (LR-PCR) as described elsewhere.[7] Briefly, 2 LR-PCRs (6.6 Kb and 8.8 Kb) were designed covering the whole *SERPINC1* gene (14 480 bp), amplicons were sequenced with nanopore technology (Oxford Nanopore Technologies [ONT], Oxford, United Kingdom) in a MinION device using barcodes and a library kit from ONT. Informatic analysis was done for single nucleotide variant and structural variant calling using an in-house pipeline. All detected variants were confirmed by Sanger sequencing. Cases with negative results were further evaluated by Sanger sequencing of the 7 exons and flanking regions, and by multiplex ligation-dependent probe amplification as indicated elsewhere.[1] Human Splicing Finder (HSF) software, version 3.1 (https://hsf.genomnis.com) was used to predict the consequences of mutations potentially affecting splicing.[8]

The AT activity was measured using an assay based on factor Xa inhibition (INNOVANCE ATIII, Siemens Healthcare Diagnostics, Marburg, Germany) or thrombin inhibition assays (Siemens Healthcare Diagnostics), the reference range for both was from 83% to 118%. The AT antigen was measured nephelometrically (Siemens Healthcare Diagnostics; reference range, 0.19–0.31 g/l).[9] AT deficiency was classified as previously described.[6]

**Results** Patient characteristics are shown in TABLE 1. A total of 8 out of 15 patients had a positive family history of VTE. The main clinical manifestation of VTE was isolated pulmonary embolism (PE; n = 5). Deep vein thrombosis (DVT) occurred in 4 patients and 2 individuals had both DVT and PE. One patient had portal vein thrombosis, one had stroke and DVT, and

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**TABLE 1** Characteristics of the study patients (continued on the next page)

| Patient ID | Sex/age, y | AT activity, % | | AT antigen level, g/l | Type of AT deficiency | Type of mutation | Location | New/reported | Clinical manifestation | Age at the first thrombo-embolic event | VTE events, n | Family history of VTE | Additional information | Current anticoagulation treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Thrombin inhibition | FXa inhibition | | | | | | | | | | | |
| 1 | F/24 | 43 | 53 | 0.14 | I | • c.175G>T<br>• p.Glu59Ter<br>• Lodz I | Exon 2 | New | No | – | 0 | 1 | NA | Heparin thromboprophylaxis in the case of trauma or surgery |
| 2 | F/23 | 60 | 76 | 0.22 | II | • c.1315C>G<br>• Pro439Ala<br>• Lodz II | Exon 6 | New | PE | 20 | 1 | 1 | Pregnancy-related PE, heterozygosity for FV Leiden | Apixaban, changed to dabigatran due to intolerance |
| 3 | F/23 | 39 | 84 | 0.229 | II | • c.1998T>G<br>• p. Phe400Val<br>• Lodz III | Exon 7 | New | DVT | 22 | 1 | 1 | Unprovoked | Rivaroxaban |
| 4 | F/20 | 55 | 62 | 0.148 | I | • c.41+3A>C<br>• Lodz IV | Intron 1 | New | DVT | 17 | 1 | 1 | Unprovoked | Rivaroxaban |
| 5 | M/48 | 73 | 80 | 0.205 | II | • Very frequent polymorphism<br>• c.1218+27C>G | Intron 6 | Reported[15] | Massive PE | 46 | 1 | 1 | Unprovoked | Acenocoumarol |
| 6 | F/26 | 69 | 69 | 0.206 | II | • c.1157T>C<br>• p.Ile386Thr | Exon 6 | Reported[14] | Cerebral venous sinus thrombosis | 21 | 1 | 0 | Myeloproliferative disorder JAK(+), hydroxycarba-mide treatment | Apixaban and ASA |
| 7 | M/48 | 41 | 54 | 0.146 | I | c.1154–14C>T | Intron 5 | Reported[1] | DVT | 32 | 2 | 1 | Sarcoidosis, obesity. First DVT event at the age of 32 and next at the age of 45, both unprovoked | Acenocoumarol and ASA |
| 8 | M/51 | 65 | 88 | 0.226 | II | No mutation | – | – | Stroke + DVT | 49 | 2 | 1 | First DVT event and then stroke 5 months later, both unprovoked | Rivaroxaban |
| 9 | F/55 | 61 | 73 | 0.194 | II | No mutation | – | – | Portal vein thrombosis | 50 | 1 | 0 | Unprovoked | Heparin thromboprophylaxis in the case of trauma or surgery |
| 10 | F/48 | 63 | 69 | 0.192 | II | No mutation | – | – | PE | 46 | 1 | 0 | Unprovoked | Dabigatran |

**TABLE 1** Characteristics of the study patients (continued from the previous page)

| Patient ID | Sex/age, y | AT activity, % | | AT antigen level, g/l | Type of AT deficiency | Type of mutation | Location | New/reported | Clinical manifestation | Age at the first thrombo-embolic event | VTE events, n | Family history of VTE | Additional information | Current anticoagulation treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Thrombin inhibition | FXa inhibition | | | | | | | | | | | |
| 11 | F/49 | 71 | 89 | 0.235 | II | No mutation | – | – | PE | 46 | 1 | 0 | Unprovoked | Rivaroxaban, and then (after epistaxis) apixaban |
| 12 | M/46 | 77 | 85 | 0.24 | II | No mutation | – | – | PE + DVT | 45 | 1 | 0 | Trauma related VTE | Rivaroxaban |
| 13 | F/55 | 79 | 83 | 0.218 | II | No mutation | – | – | DVT + PE | 52 | 2 | 0 | DVT after hormone replacement therapy and then PE after 10 months | Rivaroxaban |
| 14 | F/45 | 74 | 92 | 0.228 | II | No mutation | – | – | DVT | 31 | 1 | 1 | Unprovoked | ASA |
| 15 | M/68 | 65 | 81 | 0.203 | II | No mutation | – | – | PE | 65 | 1 | 0 | Provoked event, heterozygosity for FV Leiden | Rivaroxaban |

Abbreviations: ASA, acetylsalicylic acid; AT, antithrombin; DVT, deep vein thrombosis; F, female; FV, factor V; FXa, factor Xa; M, male; NA, not available; PE, pulmonary embolism; VTE, venous thromboembolism

one had cerebral venous sinus thrombosis. Most thromboembolic events were unprovoked (9 vs 5). The median age at first thromboembolic event was 45 years (range, 17–65 years). There were 3 patients with type I deficiency and 12 with type II deficiency. In the group of patients with type I deficiency, median AT activity and antigen level were 43% and 0.146 g/l, while in type II, 67% and 0.219 g/l, respectively. The first VTE event tended to occur earlier in patients with type I deficiency (32 years vs 41 years).

In 7 out of 15 patients, we detected mutations in the *SERPINC1* gene which can be associated with increased thromboembolic risk. Four mutations have not yet been reported in the medical literature and we named them AT Lodz I to IV.

The family history of VTE was more prevalent among carriers of a *SERPINC1* mutation than in patients with AT deficiency without *SERPINC1* defects (6/7 vs 2/8) and the first thromboembolic event occurred earlier in the former group (26 years vs 48 years; $P < 0.01$). In patients carrying a *SERPINC1* mutation, VTE was more often unprovoked than provoked (4/7 vs 2/7). Moreover, patients with detected mutation had lower AT activity levels than the remainder (median, 55% vs 68%; $P = 0.03$), but AT antigen concentrations in both groups were similar (median, 0.20 vs 0.22 g/l).

**Discussion** We identified 4 new mutations in *SERPINC1* causing AT deficiency, named AT Lodz I, II, III, and IV (**TABLE 1**).

AT Lodz I (c.175G>T in exon 2) caused a stop-gain mutation (p.Glu59Ter) that leads to a type I deficiency with no variant protein in the plasma. It was found in a 24-year-old woman free of VTE but with a positive family history of VTE. During a 16-month follow-up the woman remained asymptomatic but heparin thromboprophylaxis was prescribed in the case of trauma or surgery.

AT Lodz II (c.1315 C>G in exon 6, p.Pro439Ala) was associated with type II deficiency and was found in a 23-year-old woman with pregnancy-related PE who also carried a heterozygous factor V Leiden mutation. The patient had a positive family history of VTE. During 36 months of follow-up the woman remained asymptomatic and received long-term anticoagulation treatment with a direct oral anticoagulant (apixaban). Amino acid residue Pro439 is located at strand 4 from sheet B and it is highly conserved in the serpin superfamily. This amino acid residue was found to be mutated also to threonine (p.Pro439Thr) or leucine (p.Pro439Leu) in other patients with AT deficiency previously described. p.Pro439Thr renders an AT variant known as Antithrombin Budapest V, a type II deficiency with pleiotropic defect associated with severe thrombosis.[10]

The third new mutation detected, c.1998T>G in exon 7 leading to the missense change p.Phe400Val, was also associated with a type II deficiency and was named AT Lodz III. Moreover, the presence of aberrant AT forms in the plasma

of the proband with low heparin affinity and increased levels of the latent conformation supports a type II deficiency with pleiotropic defects.[10] Interestingly, Miyata et al[11] described another mutation in *SERPINC1*, c.1199T>C, that affected the same amino acid position (p.Phe400Ser) in a Japanese patient with AT deficiency and a positive history of DVT. It is consistent with our observation, because our 23-year-old female proband also had a positive history of unprovoked DVT. Our patient received long-term anticoagulation treatment with a direct oral anticoagulant (rivaroxaban). During a 15-month follow-up she did not have recurrence of VTE. Puurunen et al[12] described a mutation affecting the adjoining amino acid position p.His401Arg, as a result of mutation c.1202A>G in exon 6, which was also associated with AT deficiency. In all cases with mutations affecting residues Phe400 or His401, the clinical phenotype of carriers is severe, and these mutations are associated with an increased thromboembolic risk.[12]

Another new variant was an intronic mutation c.41+3A>C (intron 1) that was associated with type I deficiency. We named it AT Lodz IV. This mutation was detected in a 20-year-old woman with a positive history of unprovoked DVT (at the age of 17 years) and a positive family history of VTE. She also received rivaroxaban and remained asymptomatic during 35 months of follow-up. So far, only 27 intronic mutations have been described in acceptor or donor sequences involved in the correct splicing of *SERPINC1* (http://www.hgmd.cf.ac.uk/ac/all.php). This mutation is the fifth one affecting intron 1, with 3 of them disturbing the donor site, like this one,[13] and all of them causing type I deficiency and a severe clinical phenotype. Interestingly, similar to CS991296, the c.41+3A>C mutation does not directly affect the donor splicing sequence of exon 1, but HSF predictions strongly suggest a deleterious consequence on the donor site (10.57>6.8; –35.67%) that supports a strong effect on the correct splicing of *SERPINC1*.[1]

Two additional patients carried pathogenic *SERPINC1* defects that had been previously described: c.1157T>C responsible for a missense p.Ile386Thr change causing a type II deficiency[6,14]; and the deep intronic mutation c.1154-14C>T that caused a type I deficiency with the presence of disulphide-linked dimers of AT in the plasma.[1] One patient carried a common intronic polymorphism with unknown pathogenic consequences.[15]

Finally, 8 patients, most of them with mild AT deficiency (activity >70%) had no relevant *SERPINC1* defect detected with our methods. Further studies are required to determine if AT deficiency in these cases was acquired or caused by regulatory defects.

**Conclusion** We detected 4 new mutations in the *SERPINC1* gene causing AT deficiency, which expands our knowledge on the genetic background of AT deficiency in Poland.

## ARTICLE INFORMATION

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**CONFLICT OF INTEREST** None declared.



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