Genotype phenotype correlation in a pediatric population with antithrombin deficiency

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Abstract
Inherited antithrombin (AT) deficiency is a rare autosomal dominant disorder, caused by mutations in the AT gene (SERPINC1). Considering that the genotype phenotype relationship in AT deficiency patients remains unclear, especially in pediatric patients, the aim of our study was to evaluate genotype phenotype correlation in a Serbian pediatric population. A retrospective cohort study included 19 children younger than 18 years, from 15 Serbian families, with newly diagnosed AT deficiency. In 21% of the recruited families, mutations affecting exon 4, 5, and 6 of the SERPINC1 gene that causes type I AT deficiency were detected. In the remaining families, the mutation in exon 2 causing type II HBS (AT Budapest 3) was found. Thrombosis events were observed in 1 (33%) of those with type I, 11 (85%) of those with AT Budapest 3 in the homozygous respectively, and 1(33%) in the heterozygous form. Recurrent thrombosis was observed only in AT Budapest 3 in the homozygous form, in 27% during initial treatment of the first thrombotic event. Abdominal venous thrombosis and arterial ischemic stroke, observed in almost half of the children from the group with AT Budapest 3 in the homozygous form, were unprovoked in all cases.

Conclusion: Type II HBS (AT Budapest 3) in the homozygous form is a strong risk factor for arterial and venous thrombosis in pediatric patients.

What is Known:
• Inherited AT deficiency is a rare autosomal dominant disorder, caused by mutations in the SERPINC1 gene.
• The genotype phenotype correlation in AT deficiency patients remains unclear, especially in pediatric patients.
What is New:

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The genetic results for our paediatric population predominantly showed the presence of a single specific mutation in exon 2, that causes type II HBS deficiency (AT Budapest 3).

In this group, thrombosis mostly occurred as unprovoked, in almost half of them as abdominal thrombosis or stroke with high incidence of recurrent thrombosis, in 27% during initial treatment.

Keywords Antithrombin deficiency · SERPINC1 mutations · Pediatric population

Abbreviations

AT Antithrombin  
DVT Deep venous thrombosis  
HBS Heparin-binding site  
HGMD Human genome mutation database  
LMWH Low molecular weight heparin  
MLPA Multiplex ligation-dependent probe amplification  
PE Pulmonary embolism  
SERPINC1 Serine protease inhibitors  
TFPI Tissue factor pathway inhibitor  
VTE Venous thromboembolism  
UFH Unfractionated heparin  
VKA Vitamin K antagonist

Introduction

Inherited antithrombin (AT) deficiency is a rare autosomal dominant disorder, caused by mutations in the AT gene (SERPINC1) [14, 15], and is classified into two types. Type I is a quantitative disorder characterized by both decreased amount and activity of AT. Type II is a functional disorder classified into three subtypes according to the site of causative mutation. Thus, type II RS is caused by a defect in the AT reactive site; type II HBS is due to a mutated heparin-binding site and type II PE is related to pleiotropic mutations [1, 14, 15, 17]. The prevalence of inherited AT deficiency in the general population is estimated to be between 1:2000 and 1:3000 [29], while the prevalence of AT deficiency in patients with venous thromboembolism (VTE) is much higher, between 1:20 and 1:200 [18]. The primary role of AT in the coagulation system is inhibition of thrombin and factor Xa, but it also inhibits factors IXa, Xla, and XII. Together with the tissue factor pathway inhibitor (TFPI), AT acts as an inhibitor of FVIIa, but FVIIa only becomes sensitive to inhibition by AT when bound to tissue factor (TF) [5, 23, 25]. Congenital AT deficiency increases an individual’s risk for thrombotic complications about 30-fold (5 to 50 fold) [3, 5, 19]. The clinical presentation and severity of the disease vary depending on the type of AT deficiency and the site of mutation. The most common clinical presentation in AT deficient patients includes deep venous thrombosis (DVT) and pulmonary embolism [5, 18, 24], but the role of AT deficiency in arterial thrombosis is controversial [24, 31].

Materials and methods

From 1994 to 2018, 19 children younger than 18 years, from 15 Serbian families, with newly diagnosed AT deficiency were enrolled in the study. Thirteen of the children underwent the first thrombosis event before the age of 18, while six children were asymptomatic carriers of AT deficiency. The diagnosis of AT deficiency in asymptomatic carriers was established as a part of a family study. Originally, members of the family who had thrombosis had been examined (the first adult patient was diagnosed in 1994), and after determination of AT deficiency, the study was carried out with other family members. The investigation and diagnosis of AT deficiency, for asymptomatic presented children, was carried out between 2001 and 2018. From the moment of diagnosis, they were included in the follow-up. The age of asymptomatic subjects at the time of diagnosis ranged from 1 to 15 years. Today, the oldest asymptomatic carrier of AT deficiency is 33, while the youngest one is 5 years old.

Inclusion criteria

Inclusion criteria for pediatric patients from the Serbian AT deficiency Registery were the following: (a) age younger than or equal to 18 years at the time of AT deficiency diagnosis or thromboembolism diagnosis; (b) objective confirmation of a thrombosis event by standard imaging methods (including compression sonography, computed tomography (CT), or magnetic resonance (MR)). Exclusion criteria consisted of
liver disease, nephrotic syndrome, or lack of consent to participate in the study.

In all children, diagnosis and treatment of the thrombosis event were confirmed and conducted by pediatric hematologists. Clinical data collected from medical records for all study participants included patient demographics and disease characteristics, risk factors for thrombosis, laboratory test results, antithrombotic therapy, and outcomes. All data related to AT activity, AT antigen, and the specific mutation in the SERPINC1 gene were taken from the Registry.

Laboratory methods

AT activity

To confirm the initial diagnosis of AT deficiency revealed using the thrombin based Berichrom Antithrombin III assay (Siemens Healthcare Diagnostics, Marburg, Germany), AT activity was determined in a new sample using a chromogenic assay based on FXa inhibition in the presence of heparin (Innovance Antithrombin; Siemens Healthcare Diagnostics, Marburg, Germany). Analyses were performed on a BCS XP coagulation analyzer (Siemens Healthcare Diagnostics, Marburg, Germany), applying the reference range designated by the manufacturer, 79–111% and 83–119% respectively. In addition, the samples were tested using a second thrombin based assay (STA-Stachrom AT III, Diagnostica Stago, SAS, Asnieres sur Seine France), on a STA Compact coagulation analyzer, applying the reference range of 80–120%.

AT antigen

The antigen level was determined using the Microlatex Particle-Mediated Immunoassay, (LIATEST ATIII, Diagnostica Stago, France), applying the reference range designated by the manufacturer (80–120%).

Thrombophilia testing

All participants were tested for the presence of thrombophilia using appropriate assays on a BCS XP coagulation analyzer (Siemens Healthcare Diagnostics, Marburg, Germany). This included assessment of protein C and protein S activity, activated protein C resistance (APC-R) and the presence of lupus anticoagulant (LA).

Genetic analysis

FV Leiden and F2G20210A mutations were genotyped as previously described [2, 27].

The AT gene (gene symbol: SERPINC1, OMIM ID: 107300, and NCBI Reference Sequence: NG_012462.1) was analyzed by the gold standard Sanger fluorescent sequencing method in which all exons, exon-intron boundaries, and the promoter region were examined according to the previously described methods [21]. Fluorescent direct sequencing was carried out in an ABI PRISM 3130-Avant Genetic Analyzer (Applied Biosystems Foster City, CA), and the Sequencing Analysis 5.4 software was used for the evaluation. In cases where no causative mutations were found by Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA) was performed according to the manufacturer’s instructions using a SALSA MLPA KIT P227 (MRC-Holland, Amsterdam, the Netherlands). The MLPA products were analyzed by the Gene Mapper Software 4.1 (Thermo Fisher Scientific). Genetic variations were described according to the guidelines of the Human Genome Variation Society (HGVS, https://www.hgvs.org/).

Statistical methods

The Statistical Package for Social Sciences 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Standard statistical methods were used to evaluate frequency and percent for categorical parameters, mean and standard deviation, or median and range for ordinal or continuous scaled parameters.

Results

Genetic analysis

Confirmed by family studies and genotyping, our results showed that AT deficiency existed in three (21%) of the included families with mutations affecting exon 4, 5, and 6 of the SERPINC1 gene that causes type I AT deficiency. A mutation in exon 2 leading to type II HBS deficiency was detected in the remaining families (79%).

In the cases of type I AT deficiency, three different mutations in the heterozygous form (using NM 000488.3 as reference sequence and HGVS nomenclature for the description of mutations, where the first nucleotide of the first ATG codon is numbered as +1) c.652_654del(ATC), p.Ile218del; c.1019_1022del(TGGA); p.Leu340LeufsTer5; and c.1171C>T, p.Arg391* were encountered. The specific mutation in exon 4 leading to a frameshift and the early stop codon was associated with a thrombotic event, while carriers of the other two mutations are still asymptomatic.

Both homozygous and heterozygous forms of the specific mutation c.391 C>T, E2, p.Leu131 Phe (AT Budapest 3) that causes type II HBS AT deficiency were recorded in our study group. Analyses of prothrombotic mutations showed that one child with type II HBS in the homozygous form was a carrier of the prothrombin G20210A mutation in the heterozygous form (Table 1).
Analyses of AT activity showed that the clotting assays used for determination of AT activity had different sensitivity in cases of the type II HBS (AT Budapest 3) form. The anti-IIa-based AT activity assay (Berichrom AT) gave results in the normal range for two carriers of AT Budapest 3 in the heterozygous form. In their cases, the diagnosis of AT deficiency was based on the genetic analyses performed as part of the family study (Table 1).

**Clinical data**

Thrombosis events were observed in 13 probands: in 1/3 (33%) patients with type I, in 11/13 (85%) from the group with AT Budapest 3 in the homozygous form, and in 1/3 (33%) of those with AT Budapest 3 in the heterozygous form. In relation to asymptomatic carrier status, two subjects in each group did not develop thrombosis during the follow-up period (Table 1).

The median age of the first thrombosis for cases of AT Budapest 3 in the homozygous form was 13.1 years (range, 0.1–18), while patient 7. a. (f) from group I developed thrombosis at 14 years old and patient 6. a. (m) with Budapest 3 in the heterozygous form at the age of 12 years. Patients 3. a. (m), 6. a. (f), and 7. a. (f) had another associated risk situation at the time of thrombosis, while for homozygous carriers of AT Budapest 3, thrombosis events mostly occurred spontaneously. Thrombotic location was distal DVT in the case of type I AT deficiency. The first thrombotic events in children with AT Budapest 3 in the homozygous form were proximal DVT observed in six, abdominal vein thrombosis in three, while arterial ischemic stroke occurred in two children.

Recurrent DVT was observed only in AT Budapest 3 in the homozygous form. Five (45%) of them developed recurrent DVT, in three (27%) during initial treatment of the first thrombotic event, patients 2. a. (m), 4. a. (f), and 8. a. (f) (Table 2). They developed recurrent thrombosis during the first 2 weeks upon initial treatment of acute thrombosis. A spreading of thrombosis occurred in one, 8. a. (f), while in two remaining thrombotic processes swept the opposite leg. In patients 2. a. (m) and 4. a. (f), acute DVT was treated using UFH, with introducing of VKA within the next 24 h. Considering that thrombosis process spread despite the anticoagulant therapy, the determination of AT activity was done, and the substitution with AT concentrate was introduced in both. In patient 8. a. (f), the initial treatment involved the administration of UFH. After the thrombotic process spreading, the AT substitution was introduced and applied for 2 weeks, which was overlapped with VKA.

Most thrombotic events occurred between the ages of 10 and 18 years, except for one infant who developed abdominal thrombosis (v. cavalis inferior and v. renalis) during the second week after birth, patient 9. a. (f), previously described in a case report [11].

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**Table 1** Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Type I heterozygous</th>
<th>Type II HBS homozygous</th>
<th>Type II HBS heterozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of</td>
<td>3</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>participants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>2/1</td>
<td>6/7</td>
<td>0/3</td>
</tr>
<tr>
<td>Symptomatic (n (%))</td>
<td>1(33)</td>
<td>11(85)</td>
<td>1(33)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>12.00 (7–15)</td>
<td>13.78 (0.1–18)</td>
<td>8.33 (4–12)</td>
</tr>
<tr>
<td>(years; mean (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT activity [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(anti-IIa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>assay), ref. range</td>
<td>(79–111%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Innovance AT</td>
<td>52 (53–58)</td>
<td>19 (13–26)</td>
<td>55 (42–70)</td>
</tr>
<tr>
<td>(anti-Xa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>assay, ref. range</td>
<td>(83–119%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. STA-Stachrom AT III (anti-IIa</td>
<td>51 (49–72)</td>
<td>26 (25–29)</td>
<td>58 (52–70)</td>
</tr>
<tr>
<td>assay, ref. range</td>
<td>(80–120%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIAATEST ATIII (%)</td>
<td>59 (52–68)</td>
<td>106 (93–145)</td>
<td>101 (93–110)</td>
</tr>
<tr>
<td>mean (range), (ref. range, 80–120%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERPINC1 mutations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.652_654 del,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.Ile218 del</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heterozygous;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.1019_1022 del,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.Leu340LeufsTer5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heterozygous;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.1171 C&gt;T,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.Arg391*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heterozygous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FV Leiden/FII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G20210A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of follow-up (years) mean (range)</td>
<td>14.67 (7–22)</td>
<td>12.8 (1.8–23)</td>
<td>11.67 (4–22)</td>
</tr>
</tbody>
</table>

F, female; M, male; #, one child with type II HBS in the homozygous form was carrier of prothrombotic mutation, F2G20210A (heterozygous)

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**Coagulation tests**

Analyses of AT activity showed that the clotting assays used for determination of AT activity had different sensitivity in cases of the type II HBS (AT Budapest 3) form. The anti-IIa assay (STA-Stachrom) detected all AT Budapest 3 patients with high sensitivity. In contrast, the anti-IIa-based AT activity assay (Berichrom ATIII) gave results in the normal range for two carriers of AT Budapest 3 in the heterozygous form. In their cases, the diagnosis of AT deficiency was based on the genetic analyses performed as part of the family study (Table 1).
Table 2  Clinical characteristics in children with symptomatic thrombosis in relation to the specific genetic mutation

<table>
<thead>
<tr>
<th>Family: patient gender</th>
<th>Localization of 1st event</th>
<th>At risk situation, family history</th>
<th>1. Treatment of the first thrombosis</th>
<th>Recurrent event (year of age), localization</th>
<th>HGVS nucleotide position</th>
<th>HGVS amino acid position</th>
<th>Follow-up (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. a. (f), 17 years</td>
<td>Lower leg DVT</td>
<td>No, positive</td>
<td>1UFH/VKA for 8 months</td>
<td>19, DVT</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>23</td>
</tr>
<tr>
<td>1. b. (m), 18 years</td>
<td>Lower leg DVT</td>
<td>No, positive</td>
<td>1UFH/VKA for 6 months</td>
<td>None</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>20</td>
</tr>
<tr>
<td>2. a. (m), 13 years</td>
<td>Lower leg DVT</td>
<td>No, negative</td>
<td>1UFH/VKA for 12 months + AT substitution</td>
<td>28, abdominal under LMWH</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>19</td>
</tr>
<tr>
<td>3. a. (m), 17 years</td>
<td>Lower leg DVT</td>
<td>Infection, negative</td>
<td>1UFH/VKA for 6 months</td>
<td>None</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>20</td>
</tr>
<tr>
<td>4. a. (f), 14 years</td>
<td>Lower leg DVT</td>
<td>No, negative</td>
<td>1UFH/VKA for 12m&amp; + AT substitution</td>
<td>19, DVT</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>17</td>
</tr>
<tr>
<td>5. a. (m), 17 years</td>
<td>Stroke</td>
<td>No, positive</td>
<td>1UFH</td>
<td>77, 50, 52</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3) &amp;Death</td>
<td>29</td>
</tr>
<tr>
<td>6. a. (m), 12 years</td>
<td>Lower leg DVT</td>
<td>Surgery, positive</td>
<td>1UFH/VKA for 6 months</td>
<td>20, superficial-vein thrombosis22, superficial-vein thrombosis</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3) heterozygous</td>
<td>29</td>
</tr>
<tr>
<td>7. a. (f), 14 years</td>
<td>Lower leg DVT</td>
<td>Surgery, negative</td>
<td>1UFH/VKA for 6 months</td>
<td>None</td>
<td>E4, c.652_654 del</td>
<td>p.Ile218del heterozygous</td>
<td>8</td>
</tr>
<tr>
<td>8. a. (f), 10 years</td>
<td>Abdominal</td>
<td>No, No available data</td>
<td>1UFH + AT substitution/VKA lifelong</td>
<td>19, DVT</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>12</td>
</tr>
<tr>
<td>9. a. (f), 0.1 years</td>
<td>Abdominal</td>
<td>No, No available data</td>
<td>1UFH/LMWH + AT substitution until 6 months/VKA lifelong</td>
<td>0.1 abdominal under UFH</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3) &amp;Nephrectomy</td>
<td>9</td>
</tr>
<tr>
<td>10. a. (f), 13 years</td>
<td>Abdominal</td>
<td>No, No available data</td>
<td>1UFH + AT /VKA lifelong</td>
<td>19, DVT</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3) &amp;Nephrectomy</td>
<td>5</td>
</tr>
<tr>
<td>11. a. (m), 13 years</td>
<td>Lower leg DVT</td>
<td>No, negative</td>
<td>1UFH/VKA lifelong</td>
<td>None</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3)</td>
<td>2</td>
</tr>
<tr>
<td>12. a (f), 13 years</td>
<td>Stroke</td>
<td>No, negative</td>
<td>1LMWH/aspirin</td>
<td>54, 19, 21</td>
<td>E2, c.391 C&gt;T</td>
<td>p.Leu131Phe (AT Budapest 3) heterozygous &amp;</td>
<td>1</td>
</tr>
</tbody>
</table>

(f), female; (m), male; UFH, unfractionated heparin; VKA, vitamin K antagonist; HGVS, Human Genome Variation Society; HGMD, Human Genome Mutation Database; *(AT activity obtained with different assays); The order of the AT tests: 1, Berichrom ATIII (anti-IIa assay); ref. range (79–111%); 2, Innovance AT (anti-Xa) assay, ref. range (83–119%); 3, STA-Stachrom AT III (anti-IIa assay), ref. range (80–120%); #, F2G202 10A mutation (heterozygous); & complications and outcome
Outcome and treatment

Analyses of the first thrombotic event outcome revealed a very severe course of the disease in two patients, 9. a. (f) and 10. a. (f) with abdominal vein thromboses. Namely, thrombosis of caval and renal vein led to kidney damage with consequent nephrectomy in both cases. Acute ischemic stroke with a fatal outcome occurred in one patient 5. a. (m), while in the second patient, 12. a. (f) hemiparesis followed with weakness and impaired motility of the left hand (Table 2).

Only five subjects required AT concentrate infusions to achieve therapeutic anticoagulation, and all of them were homozygous for AT Budapest 3. In another three subjects with transient acquired risk factors, anticoagulation was discontinued after a median duration of 6 months. One of them developed superficial-vein thrombosis after 8 years of follow-up, at the age of 20. In five of the six subjects with unprovoked thrombosis, anticoagulation was discontinued after a median treatment duration of 8 months (range, 6–12). Three of these subjects have had recurrent VTE, with a median follow-up of 8 months (range, 1–24). Among the four subjects left on long-term anticoagulation therapy, none have had a VTE during the median follow-up. The last patient 12. a. (f) monitored for 12 months after ischemic stroke was switched from LMWH that was used for 3 months to anti-platelet therapy (aspirin 75 mg per day; Table 2).

Discussion

The genetic results for our study population predominantly showed the presence of a single specific mutation in exon 2 that causes type II HBS deficiency (AT Budapest 3). Comparison of the genetic findings and AT activity results obtained with different assays confirmed that Budapest 3 has different laboratory phenotype, especially in heterozygous form, where we obtained even normal results with assay 1. It is very important to be aware of possible laboratory interference of various genotypes among AT deficiency patients, in order to select an AT heparin cofactor assay with appropriate sensitivity for thrombogenic SERPINC1 variants detection, including Budapest 3 [4, 6, 8, 9, 26, 28].

Searching of the literature, we found four studies dealing with the genetics of the SERPINC gene in pediatric populations [9, 12, 13, 16]. Two of them involved children from North America and Germany and mostly included patients with AT deficiency type I [13, 16], while two other showed mostly children with type II HBS AT deficiency [9, 12]. In a brief overview, Gindele et al. presented a pediatric cohort from Hungary. The study included 32 children, from those 25 had type II HBS deficiency [9]. Khule et al. presented 5 children who were carriers of AT Budapest 3 (at the homozygous state). Four of them had severe spontaneous thromboembolic events, half of them as a newborn [12]. The North American cohort involved 29 children with AT deficiency, from those ten with VTE, in all cases provoked with no occurrence of stoke. The genetic analyses performed in 19/29 showed the predominant presence of mutations in exons 5 and 7 that cause type I AT deficiency [13]. The German study included 21 AT deficient children, among whom 76% had type I, while 24% had type II AT deficiency. In the latter group, c.391 C>T, E2, p.Leu131 Phe (AT Budapest 3) in the homozygous form was found in three cases. They also reported mutations in exons 3–6. DVT was most often present among their patients (62%), while one stroke was observed in the type II HBS group, which was similar to our study results [16].

Our study results point to some important evidence among children with AT Budapest 3 in the homozygous variant. First of all, there was a high percentage of symptomatic carriers in this group. Moreover, thrombosis events mostly occurred as unprovoked, with severe forms of clinical presentation, such as abdominal thrombosis or acute ischemic stroke in almost half of them. Likewise, the risk of recurrence is very high, considering that a quarter of the children developed rethrombosis during the initial treatment with the spread of the thrombotic process to another localization, despite anticoagulant therapy. A possible explanation is decreased sensitivity to LMWH in individuals with an HBS AT gene mutation, and the necessity for use of antithrombin concentrate to achieve adequate anticoagulation. Thromboses of abdominal veins outside the iliac-caval axis are rare but clinically relevant with serious consequences, such as liver failure, bowel infarction, or renal insufficiency [7]. In our children who had unprovoked renal vein thrombosis (RVT) and caval vein thrombosis, renal insufficiency developed, necessitating nephrectomy in both cases.

The meta-analysis of Kenet et al. showed AT deficiency to be an important associated risk factor for the development of acute ischemic stroke and central venous sinus thrombosis in children, with an OR of 7.06 (2.44–22.42) [10]. Moreover, AT deficiency has an important role in the development of ischemic stroke in young people. Thus, Martinez et al. showed that 17% of ischemic stroke cases in young adults could be attributed to a deficiency of coagulation inhibitors, half of which were due to AT deficiency [20]. However, the abovementioned studies did not include data about specific SERPINC1 mutations. It should be emphasized that genetic data on the existing mutation are very important, as we found that only homozygous carriers of the type II HBS (AT Budapest 3) developed acute ischemic stroke, unfortunately with a fatal outcome in one case. Gindele et al. [9] showed that among patients with AT deficiency only those with type II HBS deficiency developed stroke. One patient with AT Basel suffered from ischemic stroke and myocardial infarction, while two patients with AT Budapest 3 even in the heterozygous form had an ischemic stroke.
With regard to age at the first thrombotic event, we found that the critical period for the first thrombosis was between 10 and 18 years. In that period, the first thrombosis occurred in all our subjects, except for one 2-week-old baby. In the cohort of Gindele et al., one-third developed thrombosis at 0–1 years old, while two-thirds of the participants were older than 12 years [9].

Duration of anticoagulation and the need for long-term use of anticoagulants in AT deficiency patients remains unclear. The meta-analysis findings of Young et al. [32] associated AT deficiency with a threefold (95% CI, 1.43–6.33) increase in the risk of recurrent VTE. Taking into account current recommendations [22, 30], long-term anticoagulation treatment after the first VTE needs to be considered after individual risk assessment, taking into consideration the risks/benefits of such treatment. In an individual risk assessment, the AT deficiency type and SERPINC1 variant must also be taken into consideration. Our study results indicated that after the first thrombotic event all carriers of AT Budapest 3 in the homozygous form should be given a long-term anticoagulation therapy.

The limitations of our study include the relatively small number of children involved, especially for type I AT deficiency. Likewise, we did not include carriers of other mutations causing type II HBS deficiency. Additionally, as one of the limitations of our study, the fact that this is a retrospective study. In relation to that, it is possible that, in the past, VTE or PE in newborns were not diagnosed; they died before diagnosis and treatment, so it could minimize the number of newborns in our study.

In conclusion, our study results showed that AT deficiency type II HBS (AT Budapest 3) in the homozygous form is a strong risk factor for arterial and venous thrombosis in pediatric patients. The high proportion of symptomatic carriers in this group, where thrombotic events mostly occurred spontaneously, with a severe clinical presentation and a high risk of recurrence, stress the need for further research in order to improve treatment of this rare inherited thrombophilic disorder.

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Gorana Mitic: participated on the design of this study, analyzed the data, revised the manuscript critically, and agreed on the final version of this manuscript.

Branko Tomic: ran statistical analysis of the study data together with Iva Djilas and agreed on the final version of this manuscript.

Milos Kuzmanovic, Olivera Serbic and Danijela Lekovic participated on the design of this study, revised the manuscript critically, and agreed on the final version of this manuscript.

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Compliance with ethical statements

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Institutional approval for the study was granted by the Local Research Ethics Committee (EK-number 2471/1) in accordance with internationally accepted ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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