European Journal of Pediatrics (2019) 178:1471–1478
https://doi.org/10.1007/s00431-019-03433-5

ORIGINAL ARTICLE

Genotype phenotype correlation in a pediatric population with antithrombin deficiency

Mirjana Kovac[1,2] · Gorana Mitic[3] · Iva Djilas[2] · Milos Kuzmanovic[1,4] · Olivera Serbic[4] · Danijela Lekovic[1,5] · Branko Tomic[6] · Zsuzsanna Bereczky[7]

Received: 8 January 2019 / Revised: 12 July 2019 / Accepted: 16 July 2019 / Published online: 29 July 2019

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 SERPINC1gene.*
• *The genotype phenotype correlation in AT deficiency patients remains unclear, especially in pediatric patients.*
What is New:

Communicated by Peter de Winter

✉ Mirjana Kovac
mkovac008@gmail.com

Gorana Mitic
gorana_mitic@yahoo.com

Iva Djilas
ivaiva@gmail.com

Milos Kuzmanovic
mmilosbg@gmail.com

Olivera Serbic
olivera.serbic@imd.org.rs

Danijela Lekovic
danijela.lekovic@yahoo.com

Branko Tomic
kobran@imgge.bg.ac.rs

Zsuzsanna Bereczky
zsbereczky@med.unideb.hu

[1] Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[2] Blood Transfusion Institute of Serbia, Hemostasis Department, Sv. Save 39, Belgrade 11000, Serbia

[3] Institute of Laboratory Medicine, Clinical Center of Vojvodina, Faculty of Medicine Novi Sad, University of Novi Sad, Novi Sad, Serbia

[4] Institute for Health Care of Mother and Child of Serbia Dr Vukan Cupic, Belgrade, Serbia

[5] Clinic of Hematology, Clinical Center of Serbia, Belgrade, Serbia

[6] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

[7] Division of Clinical Laboratory Research, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

- *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, XIa, 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].

In unselected pediatric patients with symptomatic thromboembolism, Limperger et al. showed that the prevalence of AT deficiency adjusted for family status was 5.1% [16]. Large meta-analysis results indicate AT deficiency as one of the most important associated risks for development of the first VTE in children with an OR of 9.4 (3.34–26.66) [32]. Considering that genotype-phenotype correlation in AT deficiency patients remains unclear, investigation in this field could be helpful in better tailoring of thromboprophylaxis, especially in pediatric patients.

The aim of our study was to evaluate the clinical presentation of thrombosis in a pediatric population and its correlation to the specific mutation in the *SERPINC1* gene causing AT deficiency.

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).

Table 1 Characteristics of the study population

	Type I heterozygous	Type II HBS homozygous	Type II HBS heterozygous
Total number of participants	3	13	3
Gender (F/M)	2/1	6/7	0/3
Symptomatic (*n* (%))	1(33)	11(85)	1(33)
Age at diagnosis (years); mean (range)	12.00 (7–15)	13.78 (0.1–18)	8.33 (4–12)
AT activity [%] mean (range)			
1. Berichrom ATIII (anti-IIa assay), ref. range (79–111%)	53 (51–55)	49 (32–67)	79 (77–82)
2. Innovance AT (anti-Xa) assay, ref. range (83–119%)	52 (53–58)	19 (13–26)	55 (42–70)
3. STA-Stachrom AT III (anti-IIa assay), ref. range (80–120%)	51 (49–72)	26 (25–29)	58 (52–70)
LIATEST ATIII (%); mean (range), (ref. range, 80–120%)	59 (52–68)	106 (93–145)	101 (93–110)
SERPINC1 mutations	c.652_654 del, p.Ile218 del *heterozygous*; c.1019_1022 del, p.Leu340LeufsTer5 *heterozygous*; c.1171 C>T, p.Arg391* *heterozygous*	c.391 C>T, p.Leu131 Phe (AT *Budapest 3) homozygous#*	c.391 C>T, p.Leu131 Phe (AT *Budapest 3*) *heterozygous*
FV Leiden/*FII G20210A*	/	0/1	/
Duration of follow-up (years) mean (range)	14.67 (7–22)	12.8 (1.8–23)	11.67 (4–22)

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

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-factor Xa-based AT activity assay (Innovance AT) and 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).

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].

Table 2 Clinical characteristics in children with symptomatic thrombosis in relation to the specific genetic mutation

Family: patient/ gender age at the first event (years)	Localization of 1st event	At risk situation, family history	1. Treatment of the first thrombosis 2. Treatment of the recurrent thrombosis	AT Act* (%) A T A g (%)	Recurrent event (year of age), localization	HGVS nucleotide position	HGVS amino acid position	Follow-up (years)
1. a. (f), 17 years	Lower leg DVT	No, positive	1UFH/VKA for 8 months 2 UFH/VKA lifelong 3 UFH/AT substitution/VKA	50, 23, 29 145	19, DVT 28, abdominal under LMWH	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	23
1. b. (m), 18 years	Lower leg DVT	No, positive	1 UFH/VKA for 6 months	45, 27, 32 135	None	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	20
2. a. (m), 13 years	Lower leg DVT	No, negative	1 UFH/VKA for 12 months +AT substitution 2 LMWH/VKA lifelong	60, 32, 36, 110	0.1 DVT 28, abdominal	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	19
3. a. (m), 17 years	Lower leg DVT	Infection, negative	1 UFH/VKA for 6 months	56, 25, 32, 120	none	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	20
4. a. (f), 14 years	Lower leg DVT	No, negative	1 UFH/VKA for 12m& + AT substitution 2 UFH/VKA lifelong 3 LMWH/VKA	55, 24, 33 114	0.1, DVT under UFH 17, DVT 21, superficial-vein thrombosis under VKA	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	17
5. a. (m), 17 years	Stroke	No, positive	1 UFH	59		E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	&Death
6. a. (m), 12 years	Lower leg DVT	Surgery, positive	1 UFH/VKA for 6 months 2 LMWH for 1 months 3 LMWH for 1 months	77, 50, 52 110	20, superficial-vein thrombosis22, superficial-vein thrombosis	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) heterozygous*	29
7. a. (f), 14 years	Lower leg DVT	Surgery, negative	1 UFH/VKA for 6 months	45, 49, 50 52	None	E4, c.652_654 del	p.Ile218del *heterozygous*	8
8. a. (f), 10 years	Abdominal	No, No available data	1 UFH +AT substitution/VKA lifelong	50, 31, 36 115	0.1 abdominal under UFH	E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	12
9. a. (f), 0.1 years	Abdominal	No, positive	1 UFH/LMWH +AT substitution until 6 months/VKA lifelong	50, 19, 24 110		E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	9 &Nephrectomy
10. a. (f), 13 years	Abdominal	No, No available data	1 UFH/+AT /VKA lifelong	38, 19, 22 99		E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	5 &Nephrectomy
11. a. (m), 13 years	Lower leg DVT	No, negative	1 UFH/VKA lifelong	54, 25, 32 130		E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous*	2
12. a (f), 13 years	Stroke	No, negative	1 LMWH/aspirin	56, 19, 21 125		E2, c.391 C>T	p.Leu131Phe (AT *Budapest 3) homozygous#*	1 &Hemiparesis sin

(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%); #, *F2G20210A* 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 antiplatelet 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.

Authors' contributions Mirjana Kovac: designed the study, provided funding of the study, analyzed the data, drafted this manuscript, and agreed on the final version of this manuscript.

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: run 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.

Zsuzsanna Bereczky: provided funding of the study, revised the manuscript critically and agreed on the final version of this manuscript.

Funding This study was supported by grant 173008 from the Ministry of Education, Science and Technological Development, Serbia and from the Hungarian National Research Fund (OTKA K116228) and by the Ministry of National Economy, Hungary (GINOP-2.3.2-15-2016-00039).

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.

References

1. Alhenc-Gelas M, Plu-Bureau G, Hugon-Rodin J, Picard V, Horellou MH, GFHT study group on Genetic Thrombophilia (2017) Thrombotic risk according to SERPINC1 genotype in a large cohort of subjects with antithrombin inherited deficiency. Thromb Haemost 117(6):1040–1051
2. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67
3. Bock SC (2013) Antithrombin and the serpin family. In: Marder VJ, Aird WC, Bennett JS, Schulman S, White GC II (eds) Hemostasis and thrombosis, basic principles and clinical practice. Lippincott and Williams and Wilkin pp, Philadelphia, pp 962–972
4. Castaldo G, Cerbone AM, Guida A, Tandurella I, Ingino R, Tufano A, Ceglia C, di Minno MN, Ruocco A, di Minno G (2012) Molecular analysis and genotype-phenotype correlation in patients with antithrombin deficiency from Southern Italy. Thromb Haemost 107:673–680
5. Cooper PC, Coath F, Daly ME, Makris M (2011) The phenotypic and genetic assessment of antithrombin deficiency. Int J Lab Hematol 33:227–237
6. David D, Ribeiro S, Ferra˜o L, Gago T, Crespo F (2004) Molecular basis of inherited antithrombin deficiency in Portuguese families: identification of genetic alterations and screening for additional thrombotic risk factors. Am J Hematol 76:163–171
7. De Stefano V, Martinelli I (2012) Abdominal thromboses of splanchnic, renal and ovarian veins. Best Pract Res Clin Haematol 25:253–264
8. Gindele R, Oláh Z, Ilonczai P, Speker M, Udvari Á, Selmeczi A, Pfliegler G, Marján E, Kovács B, Boda Z, Muszbek L, Bereczky Z (2016) Founder effect is responsible for the p.Leu131Phe heparin-binding-site antithrombin mutation common in Hungary: phenotype analysis in a large cohort. J Thromb Haemost 14(4):704–715
9. Gindele R, Selmeczi A, Oláh Z, Ilonczai P, Pfliegler G, Marján E, Nemes L, Nagy Á, Losonczy H, Mitic G, Kovac M, Balogh G, Komáromi I, Schlammadinger Á, Rázsó K, Boda Z, Muszbek L, Bereczky Z (2017) Clinical and laboratory characteristics of antithrombin deficiencies: a large cohort study from a single diagnostic center. Thromb Res 160:119–128
10. Kenet G, Lu¨tkhoff LK, Albisetti M et al (2010) Stroke or cerebral sinovenous thrombosis in neonates and children. A systematic review and meta-analysis of observational studies. Circulation 121: 1838–1847
11. Kovac M, Mitic G, Jesic M, Djordjevic V, Muszbek L, Bereczky Z (2017) Early onset of abdominal venous thrombosis in a newborn with homozygous type-II HBS antithrombin deficiency. Blood Coagul Fibrinolysis 28(3):264–266
12. Kuhle S, Lane DA, Jochmanns K, Male C, Quehenberger P, Lechner K, Pabinger I (2001) Homozygous antithrombin

deficiency type II (99 Leu to Phe mutation) and childhood thromboembolism. Thromb Haemost 86(4):1007–1011
13. Kumar R, Chan AK, Dawson JE et al (2014) Clinical presentation and molecular basis of congenital antithrombin deficiency in children: a cohort study. Br J Haematol 166(1):130–139
14. Lane DA, Caso R (1989) Antithrombin: structure, genomic organization, function and inherited deficiency. Baillieres Clin Haematol 2:961–998
15. Lane DA, Olds RJ, Boisclair V et al (1993) Antithrombin III mutation database: first update. Thromb Haemost 70:361–369
16. Limperger V, Franke A, Kenet G, Holzhauer S, Picard V, Junker R, Heller C, Gille C, Manner D, Kurnik K, Knoefler R, Mesters R, Halimeh S, Nowak-Göttl U (2014) Clinical and laboratory characteristics of paediatric and adolescent index cases with venous thromboembolism and antithrombin deficiency. An observational multicentre cohort study. Thromb Haemost 112(3):478–485
17. Luxembourg B, Delev D, Geisen C, Spannagl M, Krause M, Miesbach W, Heller C, Bergmann F, Schmeink U, Grossmann R, Lindhoff-Last E, Seifried E, Oldenburg J, Pavlova A (2011) Molecular basis of antithrombin deficiency. Thromb Haemost 105:635–646
18. Maclean PS, Campbell RT (2007) Hereditary and acquired antithrombin deficiency. Drugs 67:1429–1440
19. Mahmoodi BK, Brouwer JL, Veeger NJ, van der Meer J (2008) Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age: results from a large family cohort study. Circulation 118(16):1659–1667
20. Martinez HR, Rangel-Guerra RA, Marfil LJ (1993) Ischemic stroke due to deficiency of coagulation inhibitors. Report of 10 young adults. Stroke 24:19–25
21. Maruyama K, Morishita E, Karato M et al (2013) Antithrombin deficiency in three Japanese families: one novel and two reported point mutations in the antithrombin gene. Thromb Res 132:118–123
22. Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, Vesely SK (2012) Antithrombotic therapy in neonates and children: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 141:e737S–e801S
23. Mourey L, Samama JP, Delarue M, Petitou M, Choay J, Moras D (1993) Crystal structure of cleaved bovine antithrombin III at 3.2Å resolution. J Mol Biol 232:223–241
24. Patnaik MM, Moll S (2008) Inherited antithrombin deficiency: a review. Haemophilia 14(6):1229–1239
25. Perry DJ (1994) Antithrombin and its inherited deficiencies. Blood Rev 8:37–55
26. Picard V, Bura A, Emmerich J, Alhenc-Gelas M, Biron C, Houbouyan-Reveillard LL, Molho P, Labatide-Alanore A, Sie P, Toulon P, Verdy E, Aiach M (2000) Molecular bases of antithrombin deficiency in French families: identification of seven novel mutations in the antithrombin gene. Br J Haematol 110:731–734
27. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88:3698–3703
28. Puurunen M, Salo P, Engelbarth S, Javela K, Perola M (2013) Type II Antithrombin deficiency caused by a founder mutation Pro73Leu in the Finnish population - clinical picture. J Thromb Haemost 11:1844–1849
29. Tait RC, Walker ID, Perry DJ, Islam SIAM, Daly ME, McCall F, Conkie JA, Carrell RW (1994) Prevalence of antithrombin deficiency in the healthy population. Br J Haematol 87(1):106–112
30. Tarango C, Schulman S, Betensky M, Goldenberg NA (2018) Duration of anticoagulant therapy in pediatric venous thromboembolism: current approaches and updates from randomized controlled trials. Expert Rev Hematol 11(1):37–44
31. Vossen CY, Conard J, Fontcuberta J et al (2005) Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost 3:459–464
32. Young G, Albisetti M, Bonduel M, Brandao L, Chan A, Friedrichs F, Goldenberg NA, Grabowski E, Heller C, Journeycake J, Kenet G, Krümpel A, Kurnik K, Lubetsky A, Male C, Manco-Johnson M, Mathew P, Monagle P, van Ommen H, Simioni P, Svirin P, Tormene D, Nowak-Göttl U (2008) Impact of inherited thrombophilia on venous thromboembolism in children. A systematic review and meta-analysis of observational studies. Circulation 118:1373–1382