Lack of Associations Between PAI-1 and FXIII Polymorphisms and Arterial Ischemic Stroke in Children: A Systematic Review and Meta-Analysis

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Abstract
The role of genetic risk factors for ischemic stroke seems to be in particular significance in pediatric patients. Numerous polymorphic variants of genes encoding proteins, that is, plasminogen activator inhibitor as well as coagulation factors, involved in the coagulation cascade may be related to arterial ischemic stroke (AIS) both in adults and children. We performed systematic review and 2 meta-analyses to assess possible correlations between common plasminogen activator inhibitor (PAI-1) and FXIII polymorphisms and ischemic stroke in children. We searched PubMed to identify available data published before October 2018 using appropriate keywords and inclusion criteria. Finally, 12 case–control studies were included: 8 analyzing PAI-1 polymorphism (600 children with stroke and 2152 controls) and 4—FXIII polymorphism (358 children with stroke and 451 controls). R and Comprehensive Meta-Analysis software were used to analyze the impact of the particular polymorphism in the following models: dominant, recessive, additive, and allelic. No publication bias was observed in both meta-analyses. In case of PAI-1 polymorphism, we observed no relation between 4G4G genotype of 4G allele and ischemic stroke in children. We also demonstrated lack of association between FXIII polymorphism and childhood ischemic stroke. In children with AIS, the PAI-1 and FXIII polymorphisms are not risk factors for the disease.

Keywords
arterial ischemic stroke, children, pediatric stroke, PAI-1 polymorphism, FXIII polymorphism

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Introduction
Aetiology of arterial ischemic stroke (AIS) in childhood is still not fully understood. Many risk factors are suggested to be involved in the pathogenesis of AIS in the developmental age. The most important pathological states predisposing to AIS in children are cardiac disorders, cerebral blood vessels malformations, infections, traumas, and hypercoagulable state.1 However, due to the age of pediatric patients, the role of genetic factors seems to be in particular important. Numerous polymorphic variants of genes encoding proteins involved in the coagulation cascade, lipid-related traits, or homocysteine metabolism are suggested to be related to AIS, both in adults and children.2-7 Polymorphisms within genes of plasminogen activator inhibitor (PAI-1) or coagulation factors FII, FV, FVII, and FXIII are at increasing interests.2-5

The insertion/deletion (−675_−674insG) polymorphism is located in the promoter region of PAI-1 gene and was found to increase the risk of myocardial infarction and coronary artery disease (CAD).8,9 The 4G/4G genotype was shown to increase PAI-1 activity in plasma since 4G allele binds only activator of PAI-1 transcription.10 However, some
discrepancies concerning this polymorphism can be found in the literature. Some of the authors found relations between 4G/4G genotype and PAI-1 level while others did not. Hin-dorff et al and Sarecka et al found that the 5G allele of PAI-1 gene but not the 4G allele to be a risk factor for CAD, especially in women.

According to the results obtained by Grancha et al, the influence of PAI-1 promoter 4G/5G polymorphism on PAI-1 level is more evident in a group of postmenopausal women with CAD when compared to controls (healthy women with pre- and postmenopausal status); then decrease of PAI-1 level after administration of hormone replacement therapy in CAD women correlates with 4G allele.

The role of factor XIII in cascade of clot forming and the process of fibrinolysis is crucial since FXIII plays an important role in stabilizing fibrin and increasing its resistance to fibrinolysis. Factor XIII as a zymogene is composed of 4 subunits (A2B2). The conversion to its active form (FXIIIa) occurs in the terminal phase of coagulation process in cooperation with thrombin and calcium ions. The common FXIIIa gene polymorphism, G>T transition in the second exon of FXIIIa gene results in exchange of Val to Leu substitution in A subunit. The amino acid substitution was found to be located close to a thrombin activation site. Previously, it was suggested that activity of FXIIIa was higher in Leu carriers, especially in homozygotes, while Val homozygotes presented decreased activity of this factor. However, the protective role of Leu allele, meaning the decreased risk of cardiovascular disorders, is controversial.

Studies analyzing the role of PAI-1 and FXIII polymorphisms in context to AIS in adults are common, however, show contradictory results. The study based on young Indian adults (aged up to 45 years) showed that both 4G allele and 4G/4G genotype of PAI-1 gene were associated with increased risk of ischemic stroke. On the other hand, Ranellou et al demonstrated higher frequency of the 4G/5G genotype of the PAI polymorphism in young adults with stroke than in healthy controls. The authors suggested also protective role of 5G/5G genotype. In opposite, the insertion/deletion (–675_–674insG) polymorphism within PAI-1 gene was not associated to higher risk of stroke in young Mexican adults. In case of the role of FXIII polymorphism and ischemic stroke in adults, most of the studies show no such relation while some of results suggested its possible protective role.

In contrast, little is known about the role of polymorphisms within PAI-1 and FXIII genes and AIS in children. Available data on the role of genetic polymorphisms in childhood AIS are performed most often on small groups of patients, which may affect the results, in a positive or negative way. In addition, studies may show discrepancies one to another due to the ethnic differences of the analyzed populations. Thus, systematic review can be useful to condense information about specific clinical issue while performing meta-analysis may quantitatively evaluate the analyzed problem and give more accurate results in this regard.

The aim of the present systematic review and meta-analysis was to evaluate whether PAI-1 and FXIII polymorphisms can be considered as risk factors for childhood AIS.

**Methods**

**Search Strategy**

We searched PubMed as well as the references cited in the published articles regarding the topic using appropriate keywords: “4G/5G polymorphism,” “PAI-1 polymorphism,” “SERPINE-1 polymorphism,” “FXIII polymorphism,” “Val34Leu polymorphism,” “ischemic stroke,” “cerebral infarction,” “pediatric,” “children,” “neonatal.” The searches were conducted by 2 authors (B.S.H. and I.K.) with the last search on October 2018. The following criteria were taken into account to include the study into the meta-analysis: (1) AIS diagnosed in children (in perinatal, neonatal, or childhood period), (2) AIS confirmed with computed tomography or magnetic resonance imaging, (3) case–control study, (4) full-length article written in English. Exclusion criteria were: (1) genotype or allele distributions not available; (2) conference proceedings, review articles, case reports, meta-analyses, or animal studies; (3) adult group of patients; (4) overlapping results were the basis to exclude the study from further analysis; (5) language other than English. The flow diagram shows searching process (Figure 1).

For PAI-1 polymorphism, 8 case–control studies met the inclusion criteria. A total number of 600 pediatric patients with AIS and 2152 controls were enrolled to the meta-analysis.

For FXIII polymorphism, 4 case–control studies met the inclusion criteria with a total amount of 358 pediatric patients with AIS and 451 controls. Since all included data were collected from previously published studies, no ethical issues were involved.

**Data Extraction and Methodological Quality**

The following data were extracted from each of the included studies independently by 2 authors (B.S.H. and I.K.): first author’s name, year of publication, population, age of cases and controls, size of analyzed group of patients and controls, number of particular genotype in the group, and method which was used to genotype the polymorphism. The Hardy-Weinberg equilibrium (HWE) in controls for each study according to the frequency of genotypes was calculated. The methodological quality of the studies included was examined with the use of the Newcastle-Ottawa scale for case–control studies with the scores ranged from 0 points to 11 points. We accepted study as a high quality with a score of 5 or higher. According to Minelli et al, we did not exclude studies in which distribution of genotypes deviated from HWE with a good-quality assessment. Simultaneously, low-quality researches were not excluded when no other grounds were
present. In such a situation, we performed sensitivity analysis to assess the stability of pooled odds ratio (OR) by deleting a particular study.

**Statistical Analysis**

Two authors (B.S.H. and M.S.) performed statistical analyses independently with the use of R software (version 3.3.1 with “meta” package, version 4.5-0; The R Foundation for Statistical Computing Platform) and CMA software (version 3.3.070, Bethesda, Maryland). One of the authors (M.S.) is a statistician, who supervised all statistical analyses.

The strength of association between the genetic polymorphism and AIS was determined by calculating the pooled OR with the 95% confidence interval (CI). Heterogeneity between the studies was evaluated using the Dersimonian and Laird’s Q test. When heterogeneity between the studies was significant, the pooled OR was analyzed with a random effects model, otherwise, a fixed effects model was used. The associations of studied polymorphisms with AIS were established in 4 genetic models: dominant (4G5G + 4G4G vs 5G5G for PAI-1 polymorphism and ValLeu + LeuLeu vs ValVal for FXIII polymorphism), recessive (4G4G vs 5G5G + 4G5G for PAI-1 polymorphism and LeuLeu vs ValVal + ValLeu for FXIII polymorphism), additive (4G4G vs 5G5G for PAI-1 polymorphism and LeuLeu vs ValVal for FXIII polymorphism) and allelic (4G allele vs 5G allele for PAI-1 polymorphism and Leu allele vs Val allele for FXIII polymorphism). The result was considered as statistically significant with the $P$ value below .05.

**Results**

**Study Characteristics**

Included studies analyzing PAI-1 polymorphism and FXIII polymorphism in pediatric AIS are characterized in detail in Tables 1 and 2, respectively. Genotype frequencies for PAI-1 polymorphism in controls were in agreement with HWE in 6 of the studies included. In case of PAI-1 polymorphism, the largest groups of patients with AIS.
Table 1. Characteristics of the Studies Included to the Meta-Analysis Analyzing Relation Between PAI-1 Polymorphism and AIS in Children.

| Study (Year) | Age Population | Population | Genotypes of PAI-1 4G/5G Polymorphism | Genotyping Method | HWE (for Controls; χ²; P) | Quality (Newcastle-Ottawa Scale) |
|--------------|----------------|------------|----------------------------------------|-------------------|---------------------------|--------------------------------|
| Balcerzyk et al (2011) | 8.7 ± 5.62 (years) | Poland | 70 23 35 12 | 4G4G 4G5G 5G5G | Age 7.74 ± 5.27 (years) | N 133 47 60 26 | PCR-RFLP | 0.742; .39 | 6 |
| Akar et al. (2001) | 10 months-18 years | Turkey | 43 13 20 10 | 4G4G 4G5G 5G5G | Age NS | N 113 28 57 28 | PCR-RFLP | 0.009; .92 | 5 |
| Komitopoulou et al (2006) | 2-5400 days | Greece | 87 23 50 14 | 4G4G 4G5G 5G5G | Age 3-5200 days | N 101 23 55 23 | CVD StripAssays (PCR and reverse hybridization) | 0.802; .37 | 5 |
| Nowak-Goettl et al (2001) | Mean age 4.9 years | Germany | 198 65 91 42 | 4G4G 4G5G 5G5G | Age matched to patients 9.9 ± 5.0 (years) | N 951 275 473 203 | PCR-RFLP | <0.001; .99 | 7 |
| Natesrinilkul et al (2014) | 9.8 ± 4.4 years | Taiwan | 29 2 20 7 | 4G4G 4G5G 5G5G | Age ≤18 years | N 40 1 32 7 | PCR | 16.222; <.001 | 5 |
| Coen Herak et al (2017) | 0.01-16.7 years | Croatia | 73 19 37 17 | 4G4G 4G5G 5G5G | Age ≤18 years | N 100 20 57 27 | CVD StripAssays (PCR and reverse hybridization) | 1.064; .30 | 5 |
| Miller et al (2006) | Newborns | Canada | 35 7 18 10 | 4G4G 4G5G 5G5G | Age Newborns | N 433 98 216 119 | Multilocus allele specific hybridization assay | <0.001; .99 | 6 |
| Ozyurek et al (2007) | Mean age 50 months | Turkey | 65 14 31 20 | 4G4G 4G5G 5G5G | Age NS | N 281 73 112 96 | PCR-RFLP | 10.957; <.001 | 5 |
| Total | 600 166 302 132 | Total | 2152 565 1062 529 | 4G4G 4G5G 5G5G | Genotyping Method | HWE (for Controls; χ²; P) | Quality (Newcastle-Ottawa Scale) |

Abbreviations: AIS, arterial ischemic stroke; HWE, Hardy-Weinberg equilibrium; NS, not specified; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

aChildhood AIS together with perinatal stroke.
bNeonatal arterial stroke.
cChildhood AIS together with sinovenous thrombosis.
Table 2. Characteristics of the Studies Included to the Meta-Analysis Analyzing Relation Between FXIII Polymorphism and AIS in Children.

| Study (Year)            | Age                  | Population | Genotypes of FXIII Val34Leu Polymorphism | Genotypes of FXIII Val34Leu Polymorphism | Genotyping Method | HWE (for Controls) ($\chi^2$; $P$) | Quality (Newcastle-Ottawa Scale) |
|-------------------------|----------------------|------------|----------------------------------------|-----------------------------------------|-------------------|------------------------------------|----------------------------------|
| Kopyta et al (2012)     | 8.75 ± 5.49 (years)  | Poland     | Val/Val 81                            | Val/Val 70                              | PCR-RFLP          | 0.105; 0.75                        | 6                                |
| Akar et al (2007)       | 10 months to 18 years| Turkey     | Val/Val 116                           | Val/Val 100                             | PCR-RFLP          | 0.007; 0.93                        | 5                                |
| Komitopoulou et al (2006)| 2-5400 days         | Greece     | Val/Val 88                            | Val/Val 102                             | CVD StripAssays   | 1.203; 0.27                        | 5                                |
| Coen Herak et al (2017) | 0.01-16.7 years      | Croatia    | Val/Val 73                            | Val/Val 100                            | CVD StripAssays   | 0.371; 0.54                        | 5                                |

Total 358 228 116 14 451 272 152 27

Abbreviations: AIS, arterial ischemic stroke; HWE, Hardy-Weinberg equilibrium; NS, not specified; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

*Childhood AIS together with perinatal stroke.
and controls for analysis of PAI-1 polymorphism were recruited by Nowak-Gottl et al. The study groups from the remaining studies counted less than 100 patients, of which the largest were groups of Coen Herak et al, Balcerzyk et al, and Komitopoulou et al. The largest groups of controls were again analyzed by Nowak-Gottl et al and by Miller et al. In case of FXIII polymorphism, the largest group of patients was analyzed by Akar et al, in the 3 remaining studies, number of analyzed children with AIS was comparable. The largest group of controls was recruited by Kopyta et al. To genotype the FXIII polymorphism, PCR-RFLP analysis was used in 2 of the studies and CVD StripAssays in 2 studies.

**Association Between PAI-1 Polymorphism and AIS in Children**

In case of dominant model analysis of PAI-1 4G5G+4G4G versus 5G5G, we did not observe significant heterogeneity between the analyzed studies (Cochrane Q, \( P = 0.376 \) and \( I^2 = 7.05\% \)), thus fixed effects model was used. We estimated no relation between carrier-state of 4G allele and AIS in children with pooled OR = 1.069, 95\% CI: 0.852-1.340, \( P = 0.566 \).

In case of recessive model (4G4G vs 5G5G+4G5G), we found pooled OR equal to 1.111 (95\% CI: 0.897-1.375 in fixed effects model) although the result was not significant (\( P = 0.335 \)).

Additive model (4G4G vs 5G5G) was also analyzed in fixed model with again no significance (OR: 1.165, 95\% CI: 0.888-1.529, \( P = 0.270 \)). Similarly, allelic analysis (4G vs 5G) resulted in lack of association (OR: 0.923, 95\% CI: 0.807-1.055, \( P = 0.238 \); Figure 2).

**Association Between FXIII Polymorphism and AIS in Children**

In case of dominant model analysis of FXIII polymorphism (Val-Leu+/LeuLeu vs ValVal), no significant heterogeneity between the studies included was observed (Cochrane Q \( P = 0.464 \) and \( I^2 = 0.00\% \)), thus fixed effects model was used. We estimated no relation between carrier-state of Leu allele and AIS in children with pooled OR = 0.999, 95\% CI: 0.742-1.346, \( P = 0.997 \).

In the analysis of recessive model (LeuLeu vs ValVal+ValLeu), pooled OR was equal to 0.782 (95\% CI: 0.405-1.509 in fixed effects model) although the result was not significant (\( P = 0.463 \)).

Additive model (LeuLeu vs ValVal) was also analyzed in fixed model with again no significance (OR: 0.823, 95\% CI: 0.419-1.614, \( P = 0.570 \)). Similarly, allelic analysis (Leu allele vs Val allele) resulted in lack of association (OR: 0.966, 95\% CI: 0.755-1.234, \( P = 0.779 \); Figure 3).

**Publication Bias for PAI-1 and FXIII Polymorphism**

For the PAI-1 polymorphism in all analyzed models, the Egger test did not reveal the presence of publication bias. For FXIII polymorphism again there was no publication bias (Figures 4 and 5).

**Sensitivity Analyses**

To assess the stability of the obtained results, sensitivity analysis, meaning sequential exclusion of each study included to the particular meta-analysis, was performed. We found no change in the OR value in the case of the dominant, recessive, additive, and allelic models for PAI-1 as well as FXIII polymorphism. Thus, results are stable and reliable.

**Discussion**

The obtained results based on a sizeable groups of children with AIS and healthy controls demonstrated that 4G/5G polymorphism in PAI-1 gene is not a risk factor for AIS in children. Similarly, FXIII polymorphism does not increase the risk of childhood AIS. The study of Huang et al. based on 453 female cases with first-ever stroke showed in opposite that carrier-state of 5G allele of PAI-1 polymorphism may be a protective factor for ischemic stroke (OR was equal to 0.53).

Jood et al observed a protective effect of the joint presence of PAI-1 4G/4G genotype and tissue-type plasminogen activator (tPA) CC genotype in adult patients with AIS (OR: 0.65). Similarly, Endler et al suggested that 4G/4G genotype may play a protective role in young patients with minor stroke and transient ischemic attack.

Hoeckstra et al found 4G4G genotype to be protective against stroke, transient ischemic attack, and cardiovascular mortality. According to the authors, local increase in tissue PAI-1 related to the presence of 4G allele may stabilize plaques. On the other hand, meta-analysis based on adult patients with stroke showed significant relation between 4G4G genotype and the disease (recessive model OR = 1.639). One of the earliest studies regarding the role of PAI-1 polymorphism on PAI-1 level and stroke risk was performed in elder patients and reported that healthy controls with 5G5G genotype had 36% lower plasma PAI-1 antigen levels compared to those with 4G4G genotype, while no relation between the PAI-1 polymorphism and protein level was observed in cases whose blood samples were collected less than 10 days after the stroke.

According to the authors, PAI-1 metabolism may be temporarily perturbed after a stroke. The production of PAI-1 can be stimulated by very low-density lipoprotein (VLDL), since the VLDL response site and the PAI-1 polymorphism are located next to each other.

We excluded from the present meta-analysis the study of de Paula Sabino et al performed on Brazilian patients with AIS since adolescents were analyzed together with young adults (the age of the cases was at least 11 years and below 55 years). The authors did not find the relation between the PAI-1 polymorphism and AIS, the 4G/4G genotype was significantly more frequent among controls than in cases, which also may indicate its protective role. However, increased PAI-1 plasma levels were demonstrated as a risk factor for AIS in Brazilian young patients.

Previous study also showed that patients with stroke treated with t-PA with 4G/4G genotype of PAI-1 gene had higher reocclusion rates and poor functional outcome.
Figure 2. Forrest plots of association between the PAI-1 polymorphism and arterial ischemic stroke in children in the following models: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.
Figure 3. Forrest plots of association between the FXIII polymorphism and arterial ischemic stroke in children in the following models: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.
Figure 4. Funnel plots of the 4G/5G polymorphism in the PAI-1 gene between the studies included in the meta-analysis: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.

Figure 5. Funnel plots of the G>T polymorphism (Val34Leu) in the FXIII gene between the studies included in the meta-analysis: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.
The data on the role of FXIII G>T gene polymorphism are lacking especially in pediatric population with AIS. The Turkish study performed in the year 2007 concerned the population of 116 children with AIS from 10 months to 18 years; the results of the study did not confirm the association of the polymorphism with pediatric patients with AIS. The mentioned article presents the large pediatric population with stroke examined in connection with FXIII G>T polymorphism. In the Greek pediatric population with AIS consisting of 90 patients at the age of 2 days to nearly 15 years of life and 103 controls, no association between the FXIII Val34Leu and stroke occurrence was found.23

Another case–control study performed by Kopyta et al14 concerned smaller group of pediatric patients with AIS (n = 81) with the age similar to the population analyzed by Akar et al (range from 6 months to 18 years). The results of this study did not reveal the statistical differences between the genotypes and alleles of the V34L of FXIII polymorphism between the patients and controls. Still, the difference between the L carriers in boys’ and girls’ subgroups were found. The frequency of LL and VL was much higher in girls’ group (68.6% vs 45.7%, respectively), the result was close to statistical border, but not significant (P = .04).14

In the study from Croatia, Herak et al described 73 children with AIS; within the group the patients with perinatal stroke occurring between 20th week of gestation to 28th day of life and childhood stroke between 29th day of life to 18 years of life were included.3 The perinatal group considered newborns with symptoms of AIS to 28 day of postnatal life and the group of presumed perinatal stroke when the motor symptoms resulting from “old” ischemic infarction found on neuroimaging are diagnosed retrospectively. The results obtained by Herak are in contradictory to the mentioned before on Turkish and Polish pediatric IS groups. The explanation would probably be the age of the examined children as, according to the Croatian data, the association of FXIII gene polymorphism exists but in group of childhood AIS, but not in perinatal stroke. In this capture of the problem, not only the number of the patients with AIS recruited to the research but also the age at the stroke onset matter with reference to results. On the other hand, the mentioned earlier Greek population also consisted of the newborns and the results as to the association of FXIII gene polymorphism and ischemic stroke were negative.23 All the papers point to the greater frequency of the stroke occurrence in boys’ than in girls’ subgroup. However, the explanation of the fact is obscure;

Interesting results of the influence of the specific FXIII genotype according to gender in adult patients with fatal ischemic stroke outcome were published by Hungarian authors.19 In women, the homozygous presentation of Leu34 allele was found to be the meaningful risk factor for fatal outcome of stroke leading to triple increase of the course but not in case of the stroke occurrence.19 In the other Hungarian research on the genetic risk factors for hemorrhagic stroke in adults, the Leu34Leu homozygous variant of FXIII gene polymorphism was found to be the risk factor for fatal outcome in males.39

In turn, in the Lebanon research on young stroke adults, aged 16 to 50 years, the mutations of FXIII were predominant in spinal stroke (75% of patients) and absent in patients with sinuvenous thrombosis.40 In the same study, the PAI-1 mutation was found in about half of the patients described in the article.40

Previously, PAI-1 polymorphisms was found to be associated to CAD in adults41 in contrast to FXIII polymorphism.42 In children, CAD is a very uncommon problem compared to the adult population. Also the etiology of CAD in children is completely different, but the thickness of carotid intima media and obesity are known risk factors for CAD. This knowledge should be taken into consideration in planning the strategies of CAD prevention in childhood population. Kawasaki disease (KD) is the most common and typical problem leading to coronary artery dysfunction in children. Although the problem is not very rare, especially in population below the age of 5 years, its etiology remains unclear. Previous study based on Chinese children with KD showed the association of 2 genetic polymorphisms rs16944 GG and rs1143627 AA within IL-1B gene with the risk of coronary artery lesions in children younger than 12 months, which may contribute to the pathogenesis of KD.43 However, we found no papers on CAD and PAI-1 and FXIII polymorphisms in children. In conclusion, the results of conducted systematic review along with meta-analyses based on large group of pediatric patients and controls showed that both PAI-1 and FXIII polymorphisms are not risk factors for AIS in children.

Authors’ Note
B.S.H. and I.K. were involved in conceptualization, investigation, and data curation. B.S.H. and M.S. were involved in software and formal analysis. All authors were involved in the manuscript preparation and edited and approved the final version of the manuscript.

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