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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

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discrepancies concerning this polymorphism can be found in the literature. Some of the authors found relations between 4G/4G genotype and PAI-1 level[4,5] while others did not.[11] Hindorff et al[12] and Sarecka et al[8] 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,[13] 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.[13]

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 *F13A1* 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.[14,15]

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.[16] On the other hand, Ranellou et al[17] 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.[18] In case of the role of *FXIII* polymorphism and ischemic stroke in adults, most of the studies show no such relation[17,19] while some of results suggested its possible protective role.[20]

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.[3,11,21-26] 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.[3,14,15,23] 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[27,28] 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,[29] 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

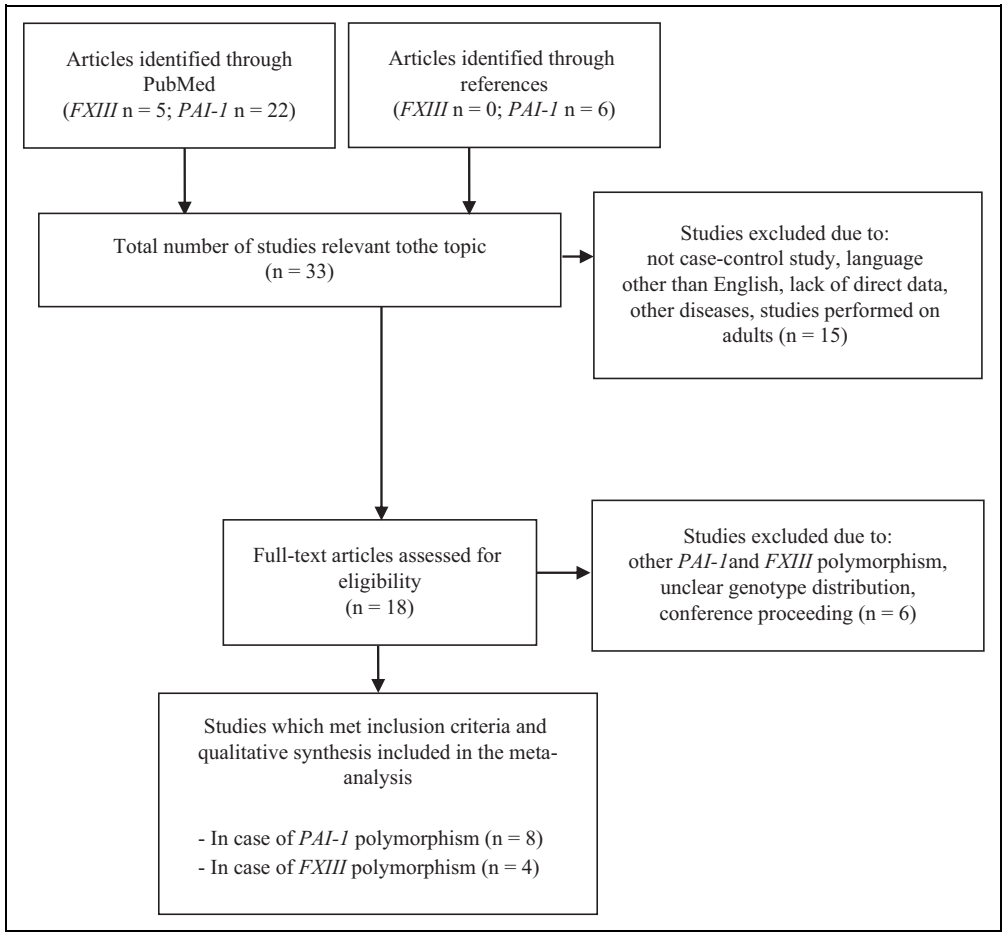

Figure 1. Flow diagram—process of searching for the articles.

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.[3,11,21-23,25] In case of *FXIII* polymorphism, distribution of genotypes in controls was in an agreement with HWE in all studies included. Caucasians were the most common race for both polymorphisms. In order to genotype the *PAI-1* polymorphism, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used in 4 of the studies,[11,21,22,26] CVD StripAssays in 2 studies,[3,23] PCR in 1 study,[24] and multilocus allele specific hybridization assay also in 1 study.[25] 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.

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

Abbreviations: AIS, arterial ischemic stroke; HWE, Hardy-Weinberg equilibrium; NS, not specified; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.
[a] Childhood AIS together with perinatal stroke.
[b] Neonatal arterial stroke.
[c] Childhood 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.

	Pediatric Patients With Arterial Ischemic Stroke						Controls							
				Genotypes of *FXIII* Val34Leu Polymorphism					Genotypes of *FXIII* Val34Leu Polymorphism					
Study (Year)	Age	Population	N	Val/Val	Val/Leu	Leu/Leu	Age	N	Val/Val	Val/Leu	Leu/Leu	Genotyping Method	HWE (for Controls) (χ^2; *P*)	Quality (Newcastle-Ottawa Scale)
Kopyta et al (2012)	8.75 ± 5.49 (years)	Poland	81	36	38	7	7.62 ± 5.62 (years)	149	70	63	16	PCR-RFLP	0.105; .75	6
Akar et al (2007)	10 months to 18 years	Turkey	116	91	25	0	Age-matched to patients	100	73	25	2	PCR-RFLP	0.007; .93	5
Komitopoulou et al (2006)	2-5400 days	Greece	88	64	22	2	3-5200 days	102	70	27	5	CVD StripAssays (PCR and reverse hybridization)	1.203; .27	5
Coen Herak et al (2017)	0.01-16.7 years[a]	Croatia	73	37	31	5	≤18 years	100	59	37	4	CVD StripAssays (PCR and reverse hybridization)	0.371; .54	5
Total			358	228	116	14	Total	451	272	152	27			

Abbreviations: AIS, arterial ischemic stroke; HWE, Hardy-Weinberg equilibrium; NS, not specified; PCR, polymerase chain reaction; RFLP, RFLP, restriction fragment length polymorphism.
[a]Childhood AIS together with perinatal stroke.

and controls for analysis of *PAI-1* polymorphism were recruited by Nowak-Gottl et al.[11] 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.[3,21,23] The largest groups of controls were again analyzed by Nowak-Gottl et al[11] and by Miller et al.[25]

In case of *FXIII* polymorphism, the largest group of patients was analyzed by Akar et al;[15] in the 3 remaining studies, number of analyzed children with AIS was comparable. The largest group of controls was recruited by Kopyta et al.[14] To genotype the *FXIII* polymorphism, PCR-RFLP analysis was used in 2 of the studies[14,15] and CVD StripAssays in 2 studies.[3,23]

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, $P = .376$ 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, $P = .566$.

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 ($P = .335$).

Additive model (4G4G vs 5G5G) was also analyzed in fixed model with again no significance (OR: 1.165, 95% CI: 0.888-1.529, $P = .270$). Similarly, allelic analysis (4G vs 5G) resulted in lack of association (OR: 0.923, 95% CI: 0.807-1.055, $P = .238$; 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 $P = .464$ 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, $P = .997$.

In the analysis of recessive model (LeuLeu vs Val-Val+ValLeu), pooled OR was equal to 0.782 (95% CI: 0.405-1.509 in fixed effects model) although the result was not significant ($P = .463$).

Additive model (LeuLeu vs ValVal) was also analyzed in fixed model with again no significance (OR: 0.823, 95% CI: 0.419-1.614, $P = .570$). Similarly, allelic analysis (Leu allele vs Val allele) resulted in lack of association (OR: 0.966, 95% CI: 0.755-1.234, $P = .779$; 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.[30] 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[31] 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.[32] Hoekstra et al found 4G4G genotype to be protective against stroke, transient ischemic attack, and cardiovascular mortality.[33] 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).[34] 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.[35] 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.[36]

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).[37] 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.[37]

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.[38]

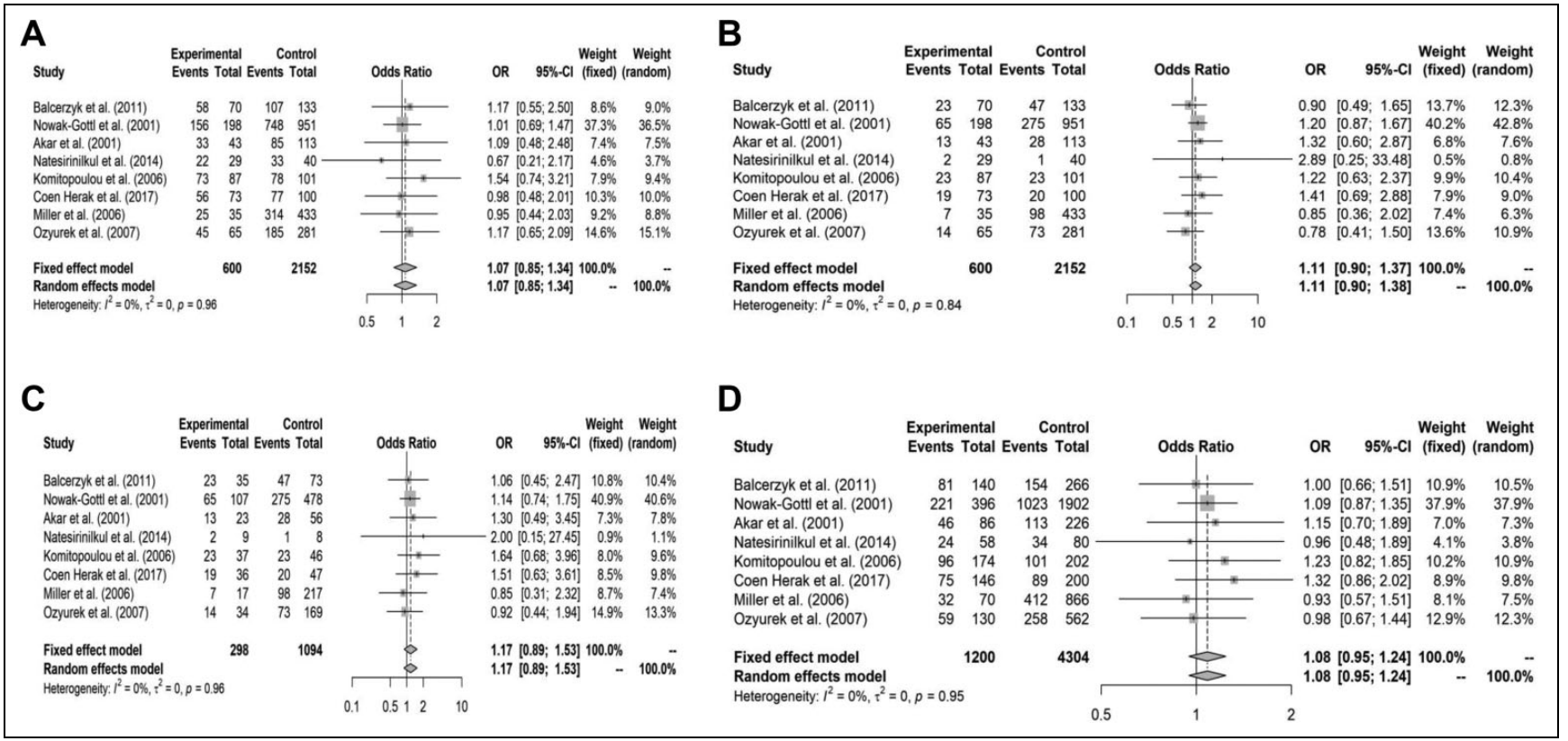

Figure 2. Forrest plots of association between the *PAI-1* polymorphism and arterial ischemic stroke in children in the following models: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.

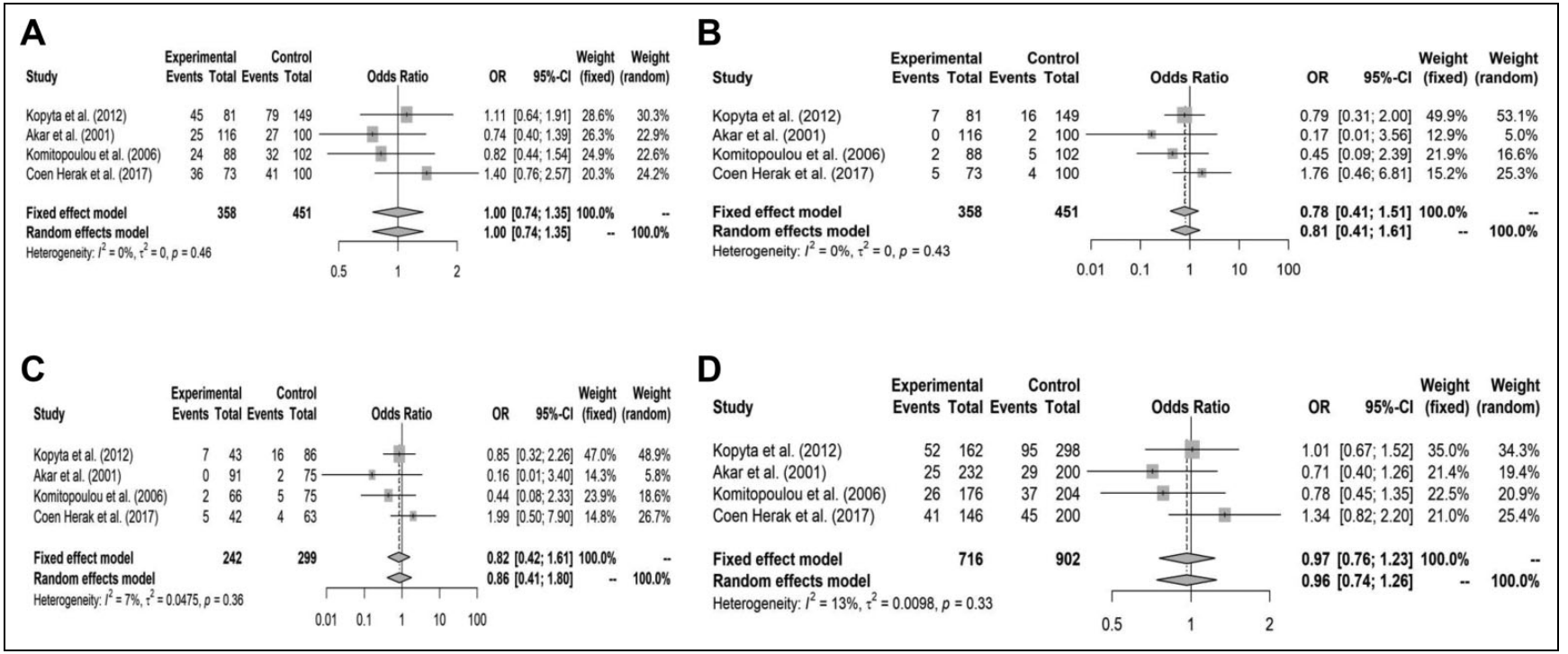

Figure 3. Forrest plots of association between the *FXIII* polymorphism and arterial ischemic stroke in children in the following models: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.

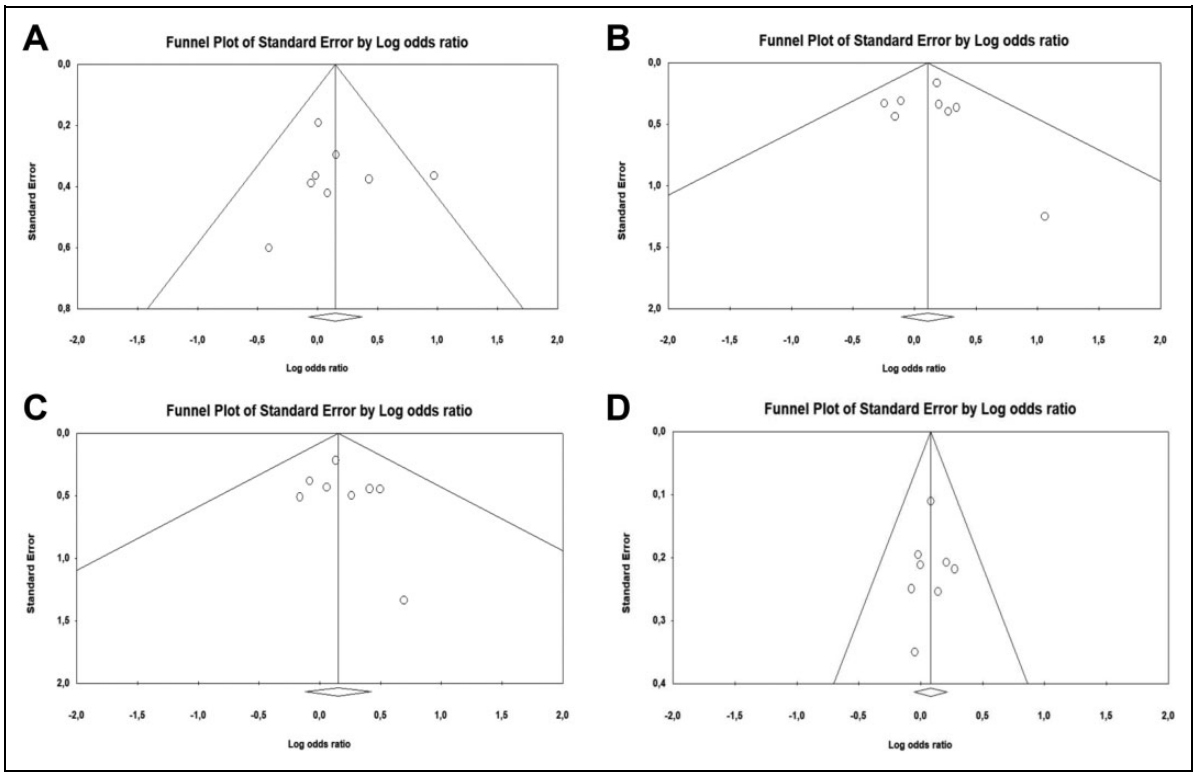

Figure 4. Funnel plots of the 4G/5G polymorphism in the *PAI-1* gene between the studies included in the meta-analysis: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.

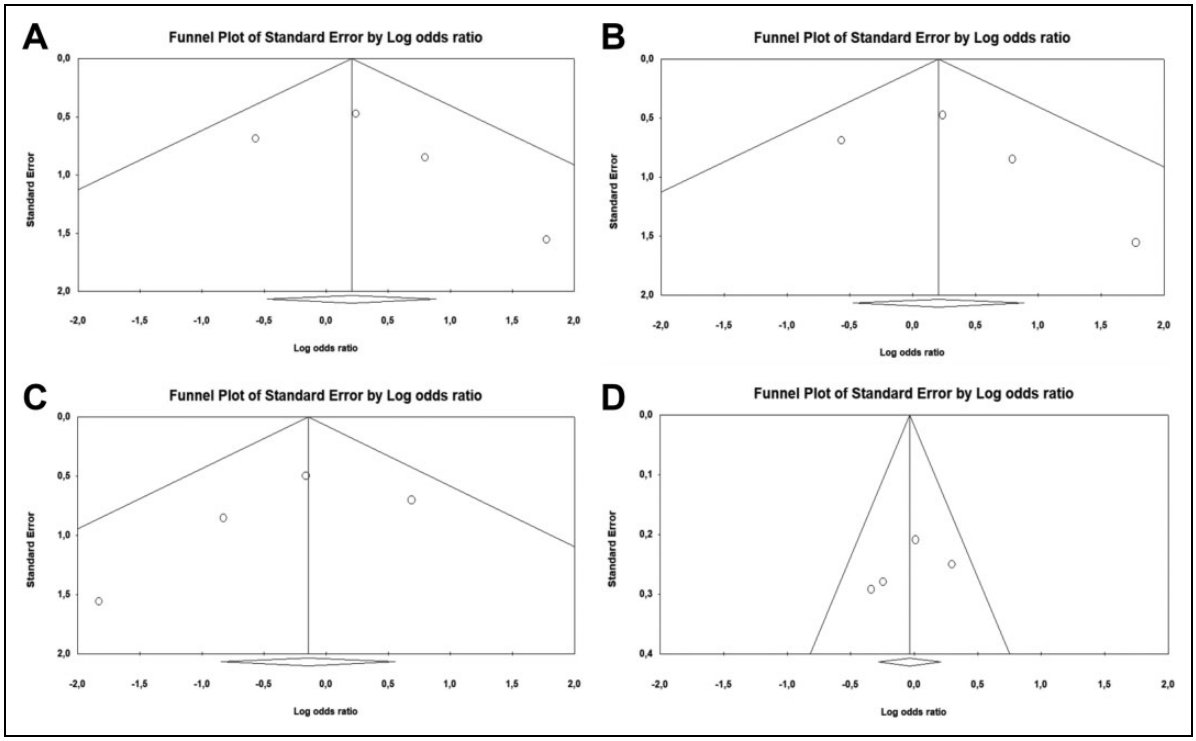

Figure 5. Funnel plots of the G>T polymorphism (Val34Leu) in the *FXIII* gene between the studies included in the meta-analysis: (A) dominant model; (B) recessive model; (C) additive model; and (D) allelic model.

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.[15] 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 al[14] 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).[15] 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.[3] 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 adults[8,41] 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.

Declaration of Conflicting Interests

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