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Genetic Aspects of Hemostasis in Children with Arterial Ischemic Stroke

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ABSTRACT

In acute and recovery periods it is obligatory to detect blood diseases and pathological conditions leading to recurrent cerebral thrombosis.

Aim of the study. To define genetic factors influencing thrombogenesis in children with AIS.

Materials and methods. At neurosurgical department of Children's City Clinical Hospital named after N.F. Filatov (St. Petersburg) we observed 33 children with AIS (20 male and 13 female) aged from 6 months to 17 years old. The diagnose was based on the developed focal neurological symptoms, that were present more than 24 hours, and changes, characteristic for AIS [18, 4], on the KT and MRT images. During history taking hemostasis disorders were detected in children and their relatives. 18 children had molecular genetic investigation of 10 genes thrombophilia markers.

Results and discussion.

In the history evidence of hemostasis disorders was found in 3 children with AIS and included: easy development and slow disappearance of bruises, inadequacy of hematomas to pattern and severity of injury, prolonged nasal, sclerotic and gum bleeding (also after traumas and operations). Parents of children with AIS hadn't suffered stroke, but their nearest relatives had different strokes – in 6 cases on the mother's side, and in 8 cases on the father's side. During history taking in some parents, mainly in mothers (15 cases), and in 2 fathers hemostasis disorders signs were detected, in the majority of cases – haemorrhagic.

At data assessment of thrombophilia genes' polymorphism (in 18 children) the occurrence frequency of mutant allele in the European population was considered. We determined the coefficient of ratio of gene mutation frequency in the study group (p%) to the mean figures in the general population (P%), and we may estimate the value of this polymorphism in the course of AIS in the study group. According to this ratio (p/P) prothrombin gene Prt (G20210A), MTHFR A1298C, fibrinogen FGB (G-455A), platelet receptor gene GP Ib and GP IIIa had the highest coefficient. The widespread cause of cerebral vessels' thrombosis, factor V Leiden mutation, was not detected.

Conclusion. The obtained results demonstrate that during history taking in children with AIS and their parents it is important to detect not only thrombosis but also haemorrhagic disorders. Children with confirmed AIS need further molecular genetic study of thrombophilic markers to detect etiological factors, as all the examined children had heterozygous and homozygous mutant genes.

Keywords: children, arterial ischaemic stroke, thrombophilia, gene markers.

INTRODUCTION

Recently researchers studying problems of children with stroke, especially arterial ischaemic stroke (AIS), pay attention to multiplicity of risk factors, directly or indirectly causing cerebrovascular diseases [8, 2, 13]. Besides congenital vascular defects, which make circulus willisii, and diseases manifesting with specific changes of vessel walls, including different connective tissue dysplasias, hemostasis disorders are described as one of the most frequent causes of thrombotic damages to cervico-cephalic vessels in children [21, 16]. In acute and recovery periods it is obligatory to detect blood diseases and pathological conditions leading to recurrent cerebral thrombosis in 7–20% of cases [15, 5, 11]. About 30% cases of AIS in children are considered idiopathic (cryptogenic) [10, 19], and it is the reason to continue search for etiological factors of this disease.

Aim of the study. To define genetic factors influencing thrombogenesis in children with AIS.

MATERIALS AND METHODS

At neurosurgical department of Children's City Clinical Hospital named after N.F. Filatov (St. Petersburg) we observed 33 children with AIS (20 male and 13 female) aged from 6 months to 17 years old. The most children were under 3 years old (21, or 63%), 1 child – from the age group 7–12 years. The boys prevailed (20 patients). Distribution by age and gender is presented in Table 1.

The diagnose was based on the developed focal neurological symptoms, that were present more than 24 hours, and changes, characteristic for AIS [18, 4], on the KT and MRT images.

Along with routine somatic and neurological assessment over time, personal background and medical history were examined, as well as risk factors of pregnancy, delivery and neonatal period. During history taking hemostasis disorders were detected in children and their families.

Detection of hemostasis disorders signs, both thrombotic and haemorrhagic, in children with AIS and their parents was fulfilled with questionnaire survey also. We searched in history for episodes of spontaneous haemorrhages of different localization under certain circumstances (for example, scleral haemorrhage, gum bleeding at teeth brushing), prolonged bleeding after operations (including tooth extraction). Also we detected nontraumatic subdermal hematomas, development of subdermal hematomas caused by minimal traumas, inadequacy of their size to pattern and severity of injury. Attention was paid to the duration disappearance duration of subdermal hematomas. All thrombosis episodes in relatives under 50 years old were registered. The mothers were questioned about prolonged profuse menstrual bleeding, excessive bleeding after abortion, and in postnatal period. Besides, we took into consideration slow wound healing in children with AIS and their parents.

Stroke pattern and extent were accessed by imaging. Computed tomography (CT) was performed in 30 children by Siemens Somatom Emotion scanner. Brain magnetic resonance imaging (MRI) findings were examined in 5 children by Toshiba excelart vantage 1.5T; in 12 children – by Hitachi «Aperto» 0.4T (T1, T2, DWI images). Seven children had phase contrast MR-angiography, 3D TOF MR-angiography was performed in 4 children.

18 children had molecular genetic investigation of 10 genes thrombophilia markers.

The results were processed with the standard programme Statistica 10.0 for Windows with criterion $\chi 2$ and Spearman rank correlation analysis.

RESULTS AND DISCUSSION

Two children had recurrent AIS, one in 6 months and the other one in 14 months after the first AIS. All other children hadn't had any thrombotic disorders before ischaemic stroke.

Clinical findings in children with AIS showed that motor disorders prevailed: hemiparesis – in 31 cases, ataxia – in 2 cases, visual impairments were the main symptoms of the disease. Minor craniocerebral trauma preceded stroke symptoms development in 23 children during different periods of time: from several minutes to 24 hours. By neuroimaging data we defined lacunar infarctions mainly in basal ganglia, thalamus and internal capsule (28 cases). In the rest 5 cases – in cortex, white matter, frontal, parietal and occipital lobes, cerebellum and brain stem. In 10 children during observation over time the secondary haemorrhage at ischaemic site was detected.

In the history evidence of hemostasis disorders in the form of haemorrhages was found in 3 children with AIS (Table 2).

Parents of children with AIS hadn't suffered stroke, but their nearest relatives had different strokes – in 6 cases on the mother's side, and in 8 cases on the father's side. During history taking we ascertained that 17 parents had haemorrhagic hemostasis disorders, the data is presented in Table 3.

Eighteen children had molecular genetic investigation of 10 genes thrombophilia markers (Table 4).

At data assessment of thrombophilia genes' polymorphism in children the occurrence frequency of mutant allele in the European population was considered. If we determine the coefficient of ratio of gene mutation frequency in the study group (p%) to the mean figures in the general population (P%), we may estimate the value of this polymorphism in the course of disease in the study group. According to this ratio (p/P) molecular genetic investigation data is presented in Table 5 in descending order.

Factor V Leiden mutation, rarely met in the European population, but in many publications standing first on the list among AIS development risk factors [6, 18], was not found in the observed children. While even more rare in this population genotypes G/g and G/A of G20210A prothrombin (1–4%) were detected in 50% of the observed patients. All the children were diagnosed with polymorphism of tissue plasminogen activator (tPA) Ins/Del gene. More than 60% of children had G/A and A/A genotypes of fibrinogen G-455A. Approximately half of the examined children had MTHFR A1298C and MTHFR C677T genes polymorphism, and 5 children were homozygous mutant allele. Homocysteine level was within normal ranges. Widespread in the European population plasminogen activator inhibitor (PAI-1) gene's mutation had mostly homozygous genotypes. The number of heterozygous genes of platelet receptors with pathological alleles 1b and A2 was also higher than general statistical data shows for European population.

DISCUSSION

Hemostasis changes play important role in ischaemic strokes pathogenesis in children. These changes have many clinical manifestations, which can be estimated only after close examination of case history, hemostasis factors dynamics and detection of symptoms group, influencing thrombogenesis.

In many recent studies there is data that AIS development factors in children are associated with blood coagulation system disorders and often are hereditary [14, 7]. That is why it is very important to detect thrombophilia signs in children, their parents and siblings. It is shown in many researches that hemorrhagic symptoms do not exclude thrombophilia

development, and in some cases are characteristic in the history of patients with thrombotic manifestations [1, 3, 20]. Such evidence demonstrates the necessity to detect in the history of children with AIS and their parents thrombosis episodes and hemorrhagic hemostasis disorders.

The obtained results (cases history) showed that only several observed children had signs of blood coagulation system disorders in the form of hemorrhages, as compared to the more frequently occurrence in their parents. This difference is likely related to the fact that parents during their life time suffered from effects of more exogenous and endogenous factors, influencing interrelations of hemostasis different components. We detected haemorrhagic signs in parents, and in other senior relatives, under 50 years old, – strokes in 8 cases. Thus, we can assume that complex, multicomponent hemostasis disorders are present, which in different situations make conditions for mainly haemorrhagic or thrombotic haemostasiopathias.

Polymorphism of genetic thrombophilic markers was noted in all the examined children with AIS, rather frequently we noted polymorphisms of the genes that have low incidence in the European population: prothrombin gene mutation G20210A, MTHFR A1298C, fibrinogen G-455A. It is well-known that namely these mutations play the most important pathogenetic role in thrombophilia development [9, 12].

CONCLUSION

The obtained results demonstrate that during history taking in children with AIS and their parents it is important to detect not only thrombosis but also haemorrhagic disorders often clinically apparent in these children. Children with confirmed AIS need further molecular genetic study of thrombophilic markers, as all the examined children had heterozygous and homozygous mutant genes. It shows the important role of genetic factors in AIS development in children, and reveals opportunities for causal and preventive treatment of thrombotic complications.

Table 1

Children's distribution by age and gender

Gender	6–12 months	1–3 years	3–7 years	7–12 years	12–18 years
Male	5	8	4	1	2
Female	4	4	3	0	2

Table 2

Clinical features of hemostasis disorders in children with AIS

Clinical features	Number of children
Easy bruises development, inadequacy to the trauma	3
Slow disappearance of bruises	3
Scleral haemorrhage	2
Epistaxis (gum bleeding)	1
Prolonged bleeding after tooth extraction	1
Prolonged bleeding after traumas, operations	1

Table 3

Clinical features of hemostasis disorders in children's relatives on mother's and father's sides

Clinical features	Mother	Father
Easy bruises development	15	1
Slow disappearance of bruises	4	1
Epistaxis (gum bleeding)	8	2
Scleral haemorrhage	6	0

Table 4

Blood analysis for thrombophilia markers' polymorphism (n = 18)

	Gene	Genotype	Number of the examined children (percent of the total number of children)	Incidence of mutant gene in European population
1.	Tissue plasminogen activator (tPA) gene	Ins/Del	18 (100%)	54%
2.	Prothrombin gene Prt (G20210A), genotype G/A	G/G (N)	9 (50%)	1–4%
		G/g	6 (33.3%)	
		G/A	3 (16.7%)	
3.	Factor V Leiden	Arg/Arg (N)	18 (100%)	2–7%
4.	Fibrinogen FGB (G-455A)	G/G (N)	7 (38.9%)	20%
		G/A	9 (50%)	
		A/A	2 (11.1%)	
5.	Integrin α-2 GPIa	C/C (N)	6 (33.3%)	35–44%
		C/T	12 (66.7%)	
6.	Plasminogen activator inhibitor gene (PAI-1)	5G/5G (N)	8 (44.4%)	26–50%
		4G/5G	5 (27.8%)	
		4G/4G	5 (27.8%)	
7.	Platelet receptor gene GP IIIa (HPA-1a/1b)	1a/1a (N)	11 (61.1%)	8–15%
		1a/1b	7 (38.9%)	
8.	Platelet receptor gene GP Ib (HPA-A1/A2)	A1/A1 (N)	10 (55.6%)	13–16%
		A1/A2	8 (44.4%)	
9.	MTHFR A1298C	A/A (N)	9 (50%)	10%
		A/C	6 (33.3%)	
		C/C	3 (16.7%)	
10.	MTHFR C677 T	C/C (N)	8 (44.4%)	32–40%
		C/T	8 (44.4%)	
		T/T	2 (11.1%)	

Table 5

Estimated value of thrombophilia genes' polymorphisms in children with AIS according to p/P ratio

Number	Gene	p/P
1.	Prothrombin Prt (G20210A), genotype G/A	12.5
2.	MTHFR A1298C	5
3.	Fibrinogen FGB (G-455A)	3.05
4.	Platelet receptor gene GP Ib (HPA-A1/A2)	2.78
5.	Platelet receptor gene GP IIIa (HPA-1a/1b)	2.59
6.	Tissue plasminogen activator (tPA) gene	1.85
7.	Integrin α-2 GPIa	1.51
8.	MTHFR C677 T	1.39
9.	Plasminogen activator inhibitor gene (PAI-1)	1.11
10.	Factor V Leiden	0

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