Features of genetic polymorphisms and clinical manifestations of primary thrombophilia in children

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Annotation. Early detection of thrombophilia is one of the main factors in the prevention of thrombotic events in the future. The presence of thrombogenic polymorphisms and their combinations determines the tactics and prognosis of the complicated course of primary thrombophilia.

Our aim was to study the features of clinical manifestations and genetic status of children with idiopathic thrombophilia, depending on the age of debut (according to the data of the multidisciplinary hospital Krasnoyarsk Regional State Budgetary Institution of Health "Krasnoyarsk Regional Clinical Center for Maternity and Childhood Protection" (KRSBIH «KRCCMCP»)).

A retrospective analysis of the case histories of children who were hospitalized at the KRSBIH «KRCCMCP» in the period 01.2014 - 01.2020 was carried out. 25 case histories of children were selected, two groups were formed.

The clinical manifestation of thrombophilia in newborns is represented mainly by venous thrombosis of the extremities in 42.86% (3 cases) or thrombosis of the renal, portal or vena cava - 42.86% (3 cases). In children aged 1 month before the age of 18, the most common vascular thrombosis of the central nervous system - 88.24% (15 patients). It was found that all patients in group 1 had a decrease in the activity of natural anticoagulants - antithrombin III and protein C. In newborns, the MTHFR gene mutation was found twice as often as in the second group (62.5% versus 29.4%, respectively). The risk of a complicated course is significantly higher in group 1,

where the RR (95% CI) was 1.821 (1.012 - 3.279). A tendency was revealed for the association of a complicated course of thrombophilia with such combinations as *MTHFR: g.677C> T, MTHFR: g.1298A> C, MTR: g.2756A> G,* and *MTRR: g.66A> G.*

Key words: thrombophilia, thrombosis, thromboembolism, hemostasis, genes.

INTRODUCTION

Thrombophilia is a hereditary or acquired condition that predisposes to pathological thrombosis [1]. Primary thrombophilia is characterized by a genetically determined tendency to form venous thromboembolic complications (VTEC) [2].

According to the literature, there are conflicting data on the incidence of this pathology in childhood, especially in the neonatal period: from 13% to 78% of all thrombosis in children are formed against the background of hereditary thrombophilia [3,4].

The urgency of the problem is also determined by the risk of a complicated course and / or re-thrombosis with disabling and lethal outcomes, which may depend on persistent risk factors such as mutations and defects in the hemostatic system [1,4,5,6].

Considering the many uncertain questions of the observation of children with primary thrombophilia, especially in the neonatal period, the study of the clinical and genetic parallels of the course and outcomes of the disease remains relevant.

During the diagnosis of primary thrombophilia, it is essential to analyze the risk factors of the disease, which are classified into clusters: A (a proven influence on the development of thrombosis in children), B (not proven, but potential risk factors), C (not proven, but possible markers) [7, 8].

Group A [9, 10, 11,12]:

▪ deficiency of natural anticoagulants (protein C, S);

▪ resistance to activated protein C;

▪ carriage of prothrombotic polymorphisms *F5: g.1691G> A, F2: g.20210G> A, MTHFR: g.677C> T*;

▪ increased concentration of lipoprotein (a);

▪ positive test for lupus anticoagulant;

▪ an increase in the titer of antiphospholipid antibodies (anti-β2-glycoprotein 1 - IgG and IgM, and anti-cardiolipin antibodies IgG and IgM).

RESULTS

In the initial sample, 25 case histories of children who were in hospital were selected according to the inclusion and exclusion criteria. One child from dichorionic twins (a girl) at the

time of examination did not have a clinic of the onset of thrombosis, while her brother had a detailed clinic of peripheral thrombosis. Since the diagnosis of hereditary thrombophilia in both children was confirmed by the presence of multiple thrombophilic mutations, aggravated by a family history of thrombotic events, the child was included in the analysis of the molecular genetic characteristics of thrombophilia in newborns.

At the beginning of the study, two age groups were formed: group 1 consisted of 8 newborn children, group 2 - 17 patients aged 28 days to 18 years. This division is due to the physiological characteristics of hemostasis in the neonatal period, which include a transient deficiency of natural anticoagulants - pC and ATIII, which is most pronounced in premature infants [13, 14].

The median gestational age of the observed group 1 was 29.8 [26.75; 38.36] weeks, 71.43% (5 people) observed were premature. The median age of patients in group 2 is 10.83 [7; 15] years (Table 1).

Table 1. Age and sex characteristics of the children under study.

Specifications		Group 1, n	Group 2, n
Sex (m/f)		4/4	7/10
Debut age	0 – 28 days	7	-
	29 days – 17 years	-	17

Note. M is male, F is female.

The clinical manifestation of the onset of thrombophilia in group 1 is represented by venous thrombosis of the extremities, as well as thrombosis of the renal, hollow, portal veins in 42.86% (3 cases each) (Table 2).

In most cases, it is difficult to identify the provoking factors for the manifestation of thrombosis. The most frequent localization of thrombosis was the vascular system of the brain with a clinical picture of transient ischemic attack (TIA) or ischemic stroke - 88.24% in group 2 (15 people).

Table 2. Clinical characteristics of the manifestation of primary thrombophilia in children.

Parameter	Group 1 (n, %)	Group 2 (n, %)	RR (95% CI)
Venous thrombosis of the upper / lower extremities	3 (42,86)	1 (5,88)	7.286 (0.906- 58.612)
Pulmonary embolism	1 (14,28)	1 (5,88)	2.429 (0.175- 33.639)
Superior / inferior vena cava / portal / mesenteric / renal vein thrombosis	3 (42,86)	-	-

Acute myocardial infarction	-	1 (5,88)	-
TIA / Ischemic stroke	1 (14,28)	15 (88,24)	0.162 (0.026- 1.002)

Note: RR (95% CI) - 95% confidence interval.

Evaluation of proven risk factors (cluster of risk factors A according to clinical guidelines) established lower values of the activity of natural anticoagulants ATIII and pC in all patients of group 1 and significantly confirmed a higher probability of their presence when calculating the risk ratio: 2.833 (1.489-5.393) for pC and 8.5 (2.312-31.247) - ATIII. When analyzing the characteristics of the genetic status of patients, it was revealed that in patients of group 1, the mutation of the *MTHFR g.677 C>T* was twice as common - 62.5% versus 29.41% in group 2 - RR (95% CI) was 2.429 (1.015-5.813), and also recorded the presence of mutations that were completely absent in the 2nd group - this is FII, and the V factor Leiden mutation (Table 3). In group 2, the detectability of the above risk factors for cluster A was significantly lower, but more often a high level of homocysteine and a decrease in pC activity were recorded.

Table 3. Proven risk factors for primary thrombophilia (risk factor cluster A).

Risk factor	Group 1		Group 2		RR (95% CI
	n	%	n	%	
Decreased pC activity*	7	100	6	35,29	2.833 (1.489-5.393)
Decreased pS activity	0	0	2	11,76	-
Decreased activity of AT III*	7	100	2	11,76	8.5 (2.312-31.247)
Increase in homocysteine	1	12,5	6	35,29	0.429 (0.062-2.949)
F5: g.1691G>A	1	12,5	0	0	-
F2: g.20210 G>A	2	25	0	0	-
MTHFR: *g.677 C>T**	5	62,5	5	29,41	2.429 (1.015-5.813)

Note: RR (95% CI) - 95% confidence interval. *p≤0,05

In group 1 patients, mutations associated with folate *(MTHFR: g.677C>T*) and methionine cycles - (*MTRR: g.66A>G* and *MTHFR: g.1298A>C*) were the most widespread (Table 4).

In group 2, the frequency of detection of the studied polymorphisms is comparable to that of newborn children. An important difference is the absence in group 2 of patients of the F5 mutation: g.1691G> A, which determines the formation of factor V resistance to one of the main physiological anticoagulants, protein C; as well as the frequency of the *MTHFR: g.677C> T* mutation, which in this group was twice as rare as in group 1.

The study of allelic variants of polymorphisms in group 1 revealed the predominance of heterozygous carriage of thrombogenic mutations in genes regulating the folate cycle - from 62.5% (5 cases) *MTRR: g.66A>G* and 37.5% (3 cases) *MTR: g. 2756A>G* and *MTHFR:*

g.677C>T (Table 4). The frequency of carriage of the homozygous state of the MTHFR variant: g.677C>T was 25% (2 cases), which corresponds to the literature data [15]. Other genes such as *MTHFR: g.1298A>C* and *MTRR: g.66A>G* had a homozygous state in 12.5% (1 case) (Table 4).

Table 4. Thrombogenic polymorphisms of hemostasis factors in primary thrombophilia.

Gene	Genome variant	Group # 1		Group # 2		RR (95% CI)
		n	%	n	%	
F7: g.10976G>A	Heterozygote	0	0	1	5,88	-
F7: g.10976G>A	Homozygote	0	0	0	0	-
F2: g.20210G>A	Heterozygote	2	25	0	0	-
F2: g.20210G>A	Homozygote	0	0	0	0	-
F5: g.1691G>A	Heterozygote	1	12,5	0	0	-
F5: g.1691G>A	Homozygote	0	0	0	0	-
MTHFR: g.1298A>C	Heterozygote	2	25	5	29,41	0.85 (0.208-3.475)
MTHFR: g.1298A>C	Homozygote	1	12,5	2	11,76	1.063 (0.112-10.067)
MTHFR: g.677C>T	Heterozygote	3	37,5	4	23,53	1.594 (0.462-5.501)
MTHFR: g.677C>T	Homozygote	2	25	1	5,88	4.25 (0.449-40.267)
MTR: g.2756A>G	Heterozygote	3	37,5	3	17,65	2.125 (0.544-8.296)
MTR: g.2756A>G	Homozygote	0	0	1	5,88	0
MTRR: g.66A>G	Heterozygote	5	62,5	5	29,41	2.125 (0.854-5.286)
MTRR: g.66A>G	Homozygote	1	12,5	6	35,29	0.354 (0.051-2.472)
ITGA2: g.807C>T	Heterozygote	1	12,5	1	5,88	2.125 (0.151-29.82)
ITGA2: g.807C>T	Homozygote	0	0	0	0	-
PAI-1: g.(-675)5g>4g	Heterozygote	1	12,5	0	0	-
PAI-1: g.(-675)5g>4g	Homozygote	0	0	1	5,88	-

Note: RR (95% CI) - 95% confidence interval.

Among the most significant polymorphisms in group 2 patients were identified: homozygous mutation *MTRR: g.66A>G* in 35.29% (6 cases), and *MTHFR: g.1298A>C* - 11.76% (2 cases). Most of the mutations in both groups are in the genes responsible for the exchange of folate, methionine and homocysteine. Polymorphisms of genes encoding key proteins of the hemostasis system were found only in the heterozygous state - in group 1 *F5: g.1691G>A* and *F2: g.20210G>A* (12.5% - 1 patient and 25% - 2 people, respectively), in group 2 *F7: g.10976G>A* - 5.88% (1 patient).

When assessing the outcomes, a tendency to the presence of a high number of disabling complications was found in both groups, which amounted to 85.72% (6 patients) and 47.06% (8 people), respectively, and more often in the neonatal group, which is confirmed by the RR

parameter (95% CI) - 1.821 (1.012 - 3.279) with a high survival rate after thrombotic events. In patients of group 2, the development of delayed complications in the form of paresis / paralysis was noted in 41.18% of cases after suffering from stroke with the following localizations of the thrombus in the basins:

- left middle cerebral artery - 4 cases (23.52%)
- right middle cerebral artery - 1 case (5.88%)
- cerebellar arteries on both sides - 1 case (5.88%)
- vertebrobasilar basin - 1 case (5.88%).

Analysis of outcomes against the background of thrombotic events (Table 5) showed that in the group of newborns, one of the two cases of mortality is due to the development of sepsis. The second case of death is associated with the development of pulmonary embolism (PE). In group 2, two deaths were recorded: the first - against the background of progressive pulmonary arterial hypertension after undergoing sarcoidosis (age 7.5 years), the second case of death of the patient due to cerebral edema against the background of acute ischemic stroke (age 10.8 years).

During the analysis of the complications of thrombosis, among the patients of our study, the following were found: conditions requiring necrectomy, paresis, paralysis, pulmonary embolism, acute renal failure, cerebral edema. In the considered cases of group 1, complications prevailed, which required the removal of necrotic tissues, and in group 2, paresis, paralysis. The likelihood of a complicated course was significantly higher in group 1, where the RR (95% CI) was 1.821 (1.012 - 3.279).

Table 5. Outcomes of the first episode of thrombosis in primary thrombophilia.

Outcome	Группа №1		Группа №2		RR (95% CI)
	n	%	n	%	
Lethal outcome	2	28,57	2	11,76	2.429 (0.422-13.993)
Full recovery	5	71,43	15	88,24	0.81 (0.491-1.334)
Complications of thrombosis*	6	85,72	8	47,06	1.821 (1.012 - 3.279)

Note: RR (95% CI) - 95% confidence interval. *p≤0.05

DISCUSSION

In group 1, according to our data, the leading criteria for confirming the diagnosis in all newborns should be considered a decrease in the activity of natural anticoagulants - ATIII and pC, less often the presence of the MTHFR mutation: g.677C> T. The features of hereditary thrombophilia in newborns include: peripheral venous disease, a high incidence of acute complications and a high mortality rate, which to a certain extent corresponds to the literature data, according to which the main manifestations of thrombophilia are: stroke (most often),

thrombosis of peripheral vessels, renal veins, and the main trigger is vascular catheterization (except for situations with stroke) [7,12].

In group 2, the predominant localization of thrombosis was the vessels of both midbrains, cerebellar and vertebrobasilar arteries; therefore, in most patients, the leading clinical syndrome was acute cerebrovascular accident of varying severity, or a clinic characteristic of a transient ischemic attack. In 58.3% of all cases (14 patients), there was a complicated course of thrombophilia, more often in the group of newborns, the total mortality rate in both groups was 16.7%, which may be associated with the presence of multiple persistent risk factors for the implementation of thrombosis.

We identified the most frequent mutations in both group: *MTHFR: g.1298A>C, MTHFR: g.677C>T, MTR: g.2756A>G, MTRR: g.66A>G*. In the second group, the genotype of patients with ischemic stroke and TIA course is represented by carriage of folate cycle genes and / or key genes of the hemostasis system, which is consistent with the literature data, which indicate a more frequent occurrence of *MTHFR: g.677C> T*, as well as a deviation from the canonical Hardy-Weinberg equilibrium for the frequencies of the MTR genotype: g.2756A> G [15].

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