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# POLYMORPHISM OF THE ORGANIC ANION TRANSPORTER PROTEIN 1B1 OATP1B1 RS2306283 GENE IN CHILDREN WITH THYROID DISEASES LIVING IN THE FAR NORTH

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The increased prevalence of iodine deficiency diseases, such as thyroid pathology, is an extremely urgent problem for Russia. Huge part of the country's territory, including the Extreme North regions, is situated in the iodine deficiency zone. Iodine deficiency diseases of the thyroid gland are widespread among prepubescent children living in this area. Polymorphism of thyroid hormone transporter genes, including the organic anion transporter 1B1 protein *OATP1B1* gene is associated with changes in their functional activity. It causes the thyroid pathologies development in children living in the conditions of iodine deficiency in the Far North. **The aim of the work** is to study the polymorphism of the organic anion transporter protein 1B1 *OATP1B1* rs2306283 gene associated with the changes of endocrine regulation in living in the Far North children with thyroid diseases. **Materials and methods**. A clinical and laboratory examination of children living in conditions of iodine deficiency in the Far North was conducted. The observation group consists of 52 children with thyroid diseases (congenital iodine deficiency syndrome, hypothyroidism, thyroid disease, endemic goiter). The comparison group includes 54 children without pathologies of the endocrine system. The iodine content in urine was determined by a unified method using spectrophotometry. The thyroid-stimulating hormone (TSH) level was determined by enzyme immunoassay. The *SNP* of the *OATP1B1* gene (rs2306283) was identified using the real-time PCR technique. **Results.** The results of the clinical and laboratory biological media analysis in the examined children demonstrate a reduced iodine content in the urine of children with thyroid pathology relative to the comparison group and the reference level ($p<0.05$). At the same time, the level of thyroid-stimulating hormone exceeds similar values in the comparison group ($p<0.05$). Polymorphism of the organic anion transporter 1B1 protein *OATP1B1* (rs2306283) gene in children with thyroid diseases is characterized by the increased C-allele frequency relative to the comparison group (OR=1.79 (CI: 1.03-3.09); $p<0.05$). It may be associated with thyroid hormone transport inhibition. **Conclusion.** Thus, the endocrine profile of children with established thyroid pathology living in conditions of iodine deficiency in the Far North is characterized by an imbalance of the pituitary-thyroid system according to the criterion of an increase in TSH content against the background of a decrease in iodine content in urine ($p<0.05$), which indicates a decrease in the functional activity of the thyroid gland. The established increased C-allele frequency of the *OATP1B1* gene (rs2306283) indicates the formation of pathogenetic trends in the transport of thyroid hormones in the presence of thyroid diseases in conditions of natural iodine deficiency. Genotyping of the *OATP1B1* gene (rs2306283) polymorphic markers associated with excessive TSH levels against the background of iodine deficiency in biological media can be used for prevention, early diagnosis and personal therapy of thyroid diseases in the population of iodine-deficient territories.

**Keywords**: gene polymorphism, thyroid gland, Far North, iodine.

**Introduction.** The increased prevalence of iodine deficiency diseases such as thyroid pathology is an extremely urgent problem for Russia due to significant part of the country's territory is situated in the iodine deficiency zone. The territory of the Far North is one of the iodine-deficient regions by the reason of permafrost, specific features of the catchment area during snowmelt, insufficient penetration of air masses from the ocean into these territories, which leads to an absolute iodine deficiency in the biosphere [11].

Iodine is a vital trace element that is necessary for normal human growth and development as an integral part of thyroid hormones – thyroxine (T4) and triiodothyronine (T3) [9]. Chronic iodine deficiency leads to the development of hypothyroidism, endemic and nodular goiter, can also cause abnormalities in the neuron development as well as mental and physical retardation in children due to inadequate production of thyroid hormones. In the territories with decreased iodine content in the environment there is an increased incidence of thyroid pathologies such as endemic goiter, hypothyroidism and other thyroid diseases associated with low iodine deficiency. Thus, in the Far North, the incidence of thyroid pathology among prepubescent children is up to 70% [12].

Polymorphic variants of genes are associated with functional changes in expression products and, as a consequence, determine an enzymatic activity level of the corresponding proteins. Therefore, different individuals may have increased resistance or excessive sensitivity to the action of damaging environmental factors, depending on the specific features of their genome and the corresponding functional activity of specific enzymes [1]. The pituitary-thyroid system of the children's population in industrial centers is characterized by an increase in activity and change in the thyroid hormones ratio in order to ensure compensatory reactions of the body in response to adverse environmental factors [4]. Consequently, polymorphism of thyroid hormone transporter genes, including the gene of the organic anion transporter protein 1B1 *OATP1B1* is associated with changes in their functional activity [6], which creates an increased risk of thyroid pathologies in children living in conditions of iodine deficiency in the Far North.

**The aim of the work** is to study the polymorphism of the organic anion transporter protein 1B1 *OATP1B1* rs2306283 gene associated with changes in endocrine regulation in living in the Far North children with thyroid diseases.

**Materials and methods.** The clinical and laboratory examination of a biological material (blood, urine, buccal epi-

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thelium) in children living in conditions of iodine deficiency in the Far North was carried out. The observation group consists of 52 children aged 4-10 years with diagnosed thyroid diseases: E00 Congenital iodine deficiency syndrome, E01 Thyroid diseases associated with iodine deficiency and similar conditions; E01.2 Goiter (endemic) associated with iodine deficiency, unspecified; E03.9 Hypothyroidism, unspecified). The comparison group includes 54 children aged 3-10 years without an established endocrine pathology. The groups are homogeneous by ethnicity and social status

The study was carried out in accordance with the norms set out in the Helsinki Declaration of the World Medical Association "Ethical principles of conducting medical research involving people as subjects" of 1975 with additions of 1983, in harmonization with the National Standard of the Russian Federation GOST-R 52379-2005 "Good Clinical Practice" (ICH E6 GCP). Parents or other legal representatives of minors have signed a voluntary informed consent for the examination of children.

Determination of iodine in urine was carried out using a unified technique on a PE-5400UF spectrophotometer ('Ekroschem', Russia). Determination of the thyroid-stimulating hormone level was carried out by enzyme immunoassay ("Hema-medica", Russia) on an Elx808 analyzer ("BioTek", USA). Genotyping by polymorphism T388C of the anion transporter protein *OATP1B1* (rs2306283) gene was carried out by PCR in real time on the CFX96 device (Bio-Rad, USA). Genetic material was extracted from buccal epithelium using the reagents set for DNA extraction from clinical material (Syntol, Russia). The human genotype was determined by the method of allelic discrimination in the specialized TaqMan program.

Methods of parametric (Student's t-test) and nonparametric (Mann-Whitney U-test) mathematical statistics using the Statistica 6.0 application software package (StatSoft, USA) were used for statistical processing of the research results. The results of the study are presented in the form of the arithmetic mean (M) and its standard error (m) studied. The analysis of associations of variant genotypes with the development of thyroid pathology was carried out using the odds ratio (OR) and its confidence interval (CI). The differences between the groups were considered statistically significant at $p<0.05$.

**Results and discussion**. The biochemical analysis results of biological media in the children's population in the Far North demonstrates that children with thyroid pathologies has significantly reduced iodine content in urine in relation to similar values of the comparison group and to the reference level ($p<0.05$). At the same time, the iodine content in the urine of children in the comparison group is comparable with the lower limit of the reference level (Table 1).

In result of the studying the indicators of endocrine regulation in the examined children by the criterion of thyroid hormone levels, it was found that the content of thyroid-stimulating hormone (TSH) in children of the observation group was 1.2 times higher than the comparison group ($p<0.05$). At the same time, the indicators of both groups are within the reference level (Table 2).

The level of thyroid-stimulating hormone in the blood accurately reflects the functional state of the thyroid gland. An increase in TSH indicates a decrease in the functional activity of the thyroid gland, which is an acute problem for children [2]. However, one of the most discussed issues in modern endocrinology both in our country and abroad is the reference lev-

**Table 1**

**Iodine content in biological media of the Far North child population**

| Indicator | Reference level | Observation group (n=52) | Comparison group (n=54) |
|---|---|---|---|
| Iodine [urine], mcg/100 cm³ | 10.00-50.00 | 6.45±1.06*/** | 9.93±2.21 |

* - differences are significant relative to the reference level ($p<0.05$);
** - differences are significant relative to the comparison group ($p<0.05$).

**Table 2**

**The content of thyroid-stimulating hormone in the blood of the Far North child population**

| Indicator | Reference level | Observation group (n=52) | Comparison group (n=54) |
|---|---|---|---|
| ТТГ, мкМЕ/см³ | 0.30-4.00 | 3.78±0.48* | 3.13±0.36 |

* - differences are significant relative to the comparison group ($p<0.05$).

**Table 3**

**The frequency genotypes and alleles distribution of *OATP1B1* gene in the Far North child population**

| Gene | Genotypes/alleles | Observation group (n=52) | Comparison group (n=54) |
|---|---|---|---|
| *OATP1B1* (rs2306283) | T/T | 0.32 | 0.45 |
| | T/C | 0.38 | 0.40 |
| | C/C | 0.30 | 0.15 |
| | T | 0.51* | 0.65 |
| | C | 0.49** | 0.35 |

Note: * - OR=0,56 (CI: 0,32-0,98);
** - OR=1,79 (CI: 1,03-3,09)

el range of thyroid-stimulating hormone. Determining of the TSH concentration is considered as the main test for assessing the functional state of the thyroid gland. Currently, the TSH level of more than 4-5 mEd/l indicates the presence of a decrease in thyroid function. The reason for the discussion was the recommendations of the National Academy of Clinical Biochemistry of the USA to reduce the upper limit of the TSH level in the blood from 4.0 to 2.0-2.5 mEd/l [8].

In result of analyzing the frequency distribution of alleles of the *OATP1B1* gene (rs2306283) in children with thyroid diseases it was established that the variant C-allele frequency in the comparison group was 1.4 times higher than in the comparison group ($p<0.05$). In the observation group, 32% of children have a homozygous wild-type TT-genotype, 38% of children are carriers of a heterozygous T/S genotype, and 30% of children had a homozygous minor CC-genotype. In the comparison group, 45% of children have a wild-type TT-genotype, 40% of children are carriers of a heterozygous T/S genotype, 15% of children have a variant homozygous C/C-genotype (Table 3).

The *OATP1B1* gene, also known as *SLCO1B1*, encodes a membrane protein that carries organic anions B1. Polypeptides transporting organic anions are a family of membrane transporters regulating cellular uptake of a number of endogenous substances and clinically important drugs. [5]. The OATP1B1 protein is one of the main absorbing transporter proteins in this group and participates in the transport of a number of endogenous and exogenous substances, including thyroid hormones [15]. More than 50 forming various haplotypes during interaction non-synonymous SNPs of this gene have been described in the scientific literature. Some of the most studied SNPs of this gene are c.521T>C/Val174Ala (rs4149056) and c.388A>G/Asn130Asp (rs2306283). These polymorphic variants of the *OATP1B1* gene are in unstable relationship with each other and are combined in different haplotypes. In some investigations the c.388G-521C haplotype is mentioned as a variant with reduced functional activity. It is associated with reduced absorption of a large number of OATP1B1 substrates *in vitro* and a noticeable increase in its concentrations in blood plasma [13, 14].

The frequency meta-analysis of the c.521T>C polymorphism of *SLCO1B1* gene in 941 people from 52 populations from Africa, the Middle East, Asia, Europe, Oceania and America revealed that this variant is widespread all over the world. However, the distribution of its frequencies in different populations is heterogeneous and varies from 1.9% in the population of Sub-Saharan Africa to 24% in the representatives group of indigenous population in America [3]. Results of similar investigation in Russia demonstrate that the TT-genotype frequency of the *SLCO1B1* gene is 64.3% in population, TC is 31.0%, CC is 4.7% [7].

**Conclusion**. Thus, the endocrine profile of children with established thyroid pathologies living in conditions of iodine deficiency in the Extreme North is characterized by an imbalance of the thyroid system. The reduced iodine content in the urine of the examined children is combined with an excessive thyrotropic hormone content, which indicates a decrease in the thyroid gland functional activity. The established increased C-allele frequency of the *OATP1B1* gene (rs2306283) indicates the formation of pathogenetic trends in the transport of thyroid hormones in the presence of thyroid diseases in conditions of natural iodine deficiency. Genotype polymorphic marker determination of the *OATP1B1* gene (rs2306283) can be used for prevention, early diagnosis and personal therapy of thyroid diseases in the population of iodine-deficient territories.

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