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Application of Molecular Genetic Research Methods to Identify Mutations in the Cyp21a2 Gene Caused by Cah in Childhood

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Abstract: The frequency of occurrence of non-classical forms of CAH in childhood is very high (1:1000), but classical forms range from 1:10,000 to 1:15,000 [7,10]. Most forms of the disease depend on the functional activity of the enzyme 21-hydroxylase. Detection of the disease using molecular genetic methods allows for quick, accurate diagnosis of the disease and selection of effective treatment for such patients.

Key words: Congenital adrenal hyperplasia, CYP21A2 gene, mutations, PCR analysis, real-time PCR analysis.

INTRODUCTION

The disease CAH was first described in the middle of the 19th century. In many literary sources, congenital adrenal hyperplasia is also defined as adrenogenital syndrome. This is due to the fact that with this disease there is a disruption in the production of major steroid hormones and, as a result, there is an accumulation of adrenocorticotropic hormone in the patient's body. The accumulation of ACTH in the patient's body leads to the development of hyperplasia. The disease is hereditary, autosomal recessive in nature [2,10]. In 90-95% of cases of CAH, disruption of hormone production is associated with defects in the synthesis of the enzyme 21-hydroxylase, which is involved in the formation of steroid hormones from cholesterol.

The clinical picture of the disease (intrauterine virilization of female fetuses, manifested by the hermaphroditic structure of the external genitalia) is explained by the fact that with 21-hydroxylase deficiency, the synthesis of adrenal androgens is not impaired. As a result, an excess of androgens leads to further virilization in girls, and to false, premature sexual development in boys. In 75% of cases, the disease manifests itself in a deficiency of mineralocarticoids, which leads to loss of salt and, without timely treatment, leads to death in the neonatal period. In the days of a child's life from 5-15, nonspecific symptoms of the disease appear, such assluggish sucking, frequent regurgitation, diarrhea, weight loss [4,7]. Without adequate early hormone replacement therapy, children die due to symptoms of acute adrenal insufficiency [1,3,5].

Clinically, deficiency of the enzyme 21-hydroxylase manifests itself in 2 forms: Classical (salt-wasting, viril) and non-classical forms.

The classic form of 21-hydroxylase deficiency leads to severe hyperandrogenism.

Table 1

Clinical forms of CAH with 21-HD deficiency

Form	Classic salt-wasting		Classic simple virile		Non-classical	
	Boy	Girl	Boy	Girl	Boy	Girl
Age at diagnosis	0–6 months	0–1 month	1.5–4 years	0 months–2 years	Postnatal period, without age delineation	
Genitals	Normal	Bisexual	Normal	Bisexual	Normal	± ↑ clitoris
Aldosterone	Reduced	Normal	Normal			
Renin	Promoted	± increased	Normal			
Cortisol	Reduced	Reduced	Normal			
17-ONP	> 600 nmol/l		>300–600 nmol/l		45–600 nmol/l (ACTH - stimulated)	
Testosterone	Promoted during pre-puberty	Promoted	Promoted during pre-puberty	Promoted	± increased in pre-puberty	± increased
Prevalence	neonatal screening					
	1:(10,000–15,000)		1:(50,000–60,000)		1:1000	
Enzyme activity (%)	0		1–2		20–50	
Treatment	Gluco- and mineralocorticoids		Glucocorticoids		Glucocorticoids (symptomatic)	

Non-classical form of CAH

The non-classical form of CAH is the most common of the autosomal recessive disorders and represents a “mild” variant of the manifestation of 21-hydroxylase deficiency [8,9].

Girls are characterized by accelerated growth, advanced bone age, premature adrenarche, pubarche and acne; clitoral hypertrophy and high posterior perineal commissure are common. Boys are also characterized by accelerated growth and growth of the penis, but there is no increase in the volume of the testicles. In the pubertal and post-pubertal period, hirsutism and dysfunction of the reproductive system are noted. Many patients have an asymptomatic course of the disease.

Purpose of the study: Due to the relevance of this problem, the purpose of the study is the molecular genetic diagnosis of 21GD deficiency and the study of the spectrum of the most common mutations of the CYP21A2 gene, leading to various forms of CAH.

Materials and methods of research.

The study was conducted on the basis of the Republican Specialized Scientific and Practical Medical Center of Endocrinology of the Ministry of Health of the Republic of Uzbekistan.

The material for the study was venous blood taken from children aged 0 to 14 years with various symptoms of androgenism. Venous blood in an amount of 1 ml was collected in 0.1 ml of sodium citrate solution (anticoagulant) and stored at a temperature of -20 0C.

During the study, a DNA molecule was isolated from the obtained venous blood, which was then used in PCR analysis to identify the active CYP21A2 gene. At the next stage, real-time PCR analysis was carried out to determine the most common mutations of the CYP21A2 gene caused by CAH.

All examined children underwent molecular genetic analysis for the presence of 4 mutations (C89T, T999A, C1994T andG1683T) of the CYP21A2 gene.

Research results.

As a result of the research, biological material (venous blood) was collected from 50 children aged 0 to 14 yearswith hyperandrogenic symptoms. INDNA was isolated from these samples. The quality and quantity of isolated genomic DNA were assessed. A two-stage PCR analysis was performed. At the first stage, a locus-specific PCR analysis was carried out; at the second stage, an allele-specific real-time PCR analysis was carried out to determine 4 mutations C89T, T999A, C1994T and G1683T in the CYP 21A2 gene.

Molecular genetic analysis of the CYP21A2 gene, carried out in 50 children, revealed 21-HD deficiency in 14 cases, which served as the basis for the diagnosis of CAH.

The analyzes revealed 3 mutations of the CYP21A2 gene out of 4 studied mutations:S89T, T999A, G1683T and C1994T. Of the 50 samples studied, mutations were identified in 9 samples, which accounted for 18% of the total. We also identified cases of several mutations in one patient in the CYP21A2 gene.

The C1994T mutation was detected in 4 children, which is 8% of the total number of children examined. Of these, one was homozygous, and the remaining 3 were heterozygous. The T999A mutation was detected in 4 of the selected samples, amounting to 8%. It should be noted that in 2 out of 4 cases the identified mutations were homozygous, the remaining 2 were heterozygous. The heterozygous G1683T mutation was detected in one sample, accounting for 2%.

The C89T mutation of the CYP21A2 gene was not detected in any sample.

Based on the studies conducted, it can be assumed that it is necessary to use point mutations of the CYP21A2 gene.

Conclusions. Based on the results of the work done, we can conclude that in 50 examined children with various symptoms of androgenism, of the 4 mutations we studied, three mutations were most common, which amounted to 18% of the total number of examined children.

The most common mutations of the 4 mutations of the CYP21A2 gene we studied in children in the Uzbek region are mutations T999A (8%) and C1994T (8%).

The conducted molecular genetic study indicates its usefulness for identifying mutations in the CYP21A2 gene in children, both for choosing treatment tactics for children in the postnatal and pubertal period, and for preventing the hereditary transmission of various forms of CAH in the future.

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