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"The significance of the GNRH 1 gene polymorphism (rs 6185, rs1812594) in the development of idiopathic hypogonadotropic hypogonadism."

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Annotation
The purpose of the study is to study the significance of the GNRH 1 gene polymorphism (rs 6185, rs1812594) in the development of idiopathic hypogonadotropic hypogonadism in boys and girls.
Material and research methods. To achieve this goal, a genetic study was conducted in 90 adolescents diagnosed with iHH, selected during screening in pilot regions of the Republic of Uzbekistan as part of an applied project in the period June-August 2023: Kashkadarya, Jizzakh, Surkhandarya, Namangan regions and the Republic of Karakalpakstan. Among the 90 individuals, there were 73 boys and 17 girls, with an average age of 14.3 years.
Research results. The most common patients among those examined were aged 14.7 years - 22 boys and 8 girls (IV stage according to Tanner by age). Moreover, the stage of puberty upon examination corresponded to II in both 22 boys (30.1%) and 8 girls (47%).
In general, when assessing the stage of puberty, it was revealed that 30 adolescents had delayed puberty, that is, puberty corresponded to stage 2 at the age of 13-14 years in both boys and girls. At the same time, in group 1 of patients with nIH+ hypothyroidism, the lowest average values of height and weight were observed in comparison with group 1 of patients with nIHH.
Conclusions. Our results confirm that the GNRH 1 gene polymorphism is the genetic cause of nIHH. At the same time, out of 90 patients with clinical and hormonal data of nIHH, the rs 6185 polymorphism of the GNRH 1 gene was found in 5 (6.6%) cases and rs1812594 was found in (4.4%) cases.

Key words: hypogonadism, adolescents, GNRH 1

Topicality. Gonadotropin-releasing hormone (GnRH) is the main hormone of the reproductive endocrine system. The existence of central hormones that regulate reproduction was postulated a century ago [1]. In 1910, Crowe et al. [2]. have been shown that disruption of the hypothalamic-pituitary connection in dogs prevents the onset of puberty. Subsequent studies have led to the hypothesis that the pituitary gland is controlled by a hypothalamic factor [3-6]. However, it wasn't until 1971 that the amino acid The sequence of GnRH was determined after extraction from the hypothalamus of thousands of pigs and sheep by the Schally and Guillemin groups [7, 8].

GnRH-secreting neurons are known to originate in the olfactory bulb and migrate to the hypothalamus [1]. Once in the hypothalamus, these GnRH neurons project axons toward the median elevation and synchronize GnRH secretion in a pulsating manner. GnRH is then carried by portal circulation to the pituitary gland and stimulates anterior pituitary gonadotropin hormones that secrete gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins then induce steroidogenesis and gametogenesis in the gonads.

In addition to extensive physiological studies of the central role of GnRH in reproduction [1], human genetic studies have highlighted the critical role of GnRH in the regulation of reproduction [9,10]. Idiopathic

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hypogonadotropic hypogonadism (IGG) is characterized by the absence of spontaneous sexual development against the background of low levels of sex steroids and gonadotropins with normal pituitary function. When combined with anosmia, this hypogonadotropism is called Kallmann syndrome (CS), whereas isolated hypogonadotropic hypogonadotropism with a normal sense of smell is called normosmic IGG (). Studies of patients with nIGGG and KS have led to the identification of several genes that regulate reproduction. Mutations *in KAL1* [11,12], FGFR1 *[13], FGF8* [14], PROK2 [15], PROKR2 *and CHD7* [*16]* are thought to disrupt the development and migration of GnRH neurons, leading to SC and/or. Patients with mutations in *PCSK1*, which encodes the prohormone convertase 1/3, exhibit hypogonadotropic hypogonadism due to abnormal processing of the GnRH decapeptide from its precursor prohormone [17]. Mutations in *GPR54* cause by disrupting normal GnRH secretion [18. 19], and mutations in *GNRHR*, which encodes the GnRH receptor, lead to an inability to respond to GnRH [20]. Mutations in the *TAC3 and* TACR3 genes, *which encode neurokinin B and its receptor, respectively, have recently been implicated in [21], although their exact functions in reproduction remain unclear.*

A big omission in the list of genes involved in IGG *is GNRH1 itself , which encodes a preprohormone that is eventually processed to form GnRH. The results obtained in mice definitely suggest that* GNRH1 mutations *in humans can cause*. The hpg mouse carries a *Gnrh1* deletion , which arose spontaneously and leads to a complete lack of GnRH synthesis [22, 23]. Male and female *hpg mice* are sexually infantile, infertile, and have low levels of sex steroids and gonadotropins [22]. In one of the earliest demonstrations of successful gene therapy, the reproductive deficiency of *hpg* mice was eliminated with the *Gnrh1* transgene [24]. With the exception of reproductive phenotypes, *hpg mice* appear to be perfectly normal, although dental abnormalities have recently been reported [25]. The clear association *of loss of Gnrh1* function in mice with hypogonadotropic hypogonadism makes the absence *of GNRH1* mutations in humans as a cause of nIHH even more puzzling.

GNRH1 is an obvious candidate gene for, so why haven't mutations been identified in *GNRH1 yet?* [26]. One possibility is that functional mutations in genes encoding ligands occur less frequently than in genes encoding receptors due to differences in the size of ligands and related receptors. Encoding a peptide product consisting of only 92 amino acids, GNRH1 *represents a smaller "target" for mutations than the 328 amino acids encoded* by GNRHR . Indeed, for other ligand-receptor pairs involved in/KS, fewer mutations were recorded in genes encoding ligands (*FGF8* and *PROK2*) than in genes encoding receptors [9, 10]. An alternative explanation for the rarity of *GNRH1* mutations is that they are rapidly eliminated from the population. This may be due to an inability to pass on mutations to future generations, as would be expected from mutations that cause fertility declines.

Idiopathic hypogonadotropic hypogonadism (IGG) is a condition characterized by the absence of puberty with low levels of sex steroids and gonadotropins. IGG occurs due to abnormal secretion or action of the main reproductive hormone, gonadotropin-releasing hormone (GnRH). Several genes have been found to be mutated in IGG patients, but to date, no mutations have been identified in the most obvious candidate gene, *GNRH1* itself , which encodes a preprohormone that is eventually processed to form GnRH.

All of the above underscores the relevance of this study and the reason for it.

To this end, we tested the DNA of 90 patients with normosmic IGG () and 20 healthy control subjects for changes in the GNRH1 sequence.

The aim of the study was to study the significance of GNRH 1 gene polymorphism (rs 6185, rs1812594) in the development of normosmic IGG in boys and girls.

Material and methods of research. To achieve this goal, a genetic study was carried out in 90 adolescents with diagnoses of, selected during screening in pilot regions of the Republic of Uzbekistan as part of an applied project in the period June-August 2023: Kashkadarya, Jizzakh, Surkhandarya, Namangan regions and the Republic of Karakalpakstan. Of the 90 individuals, there were 73 boys and 17 girls, with an average age of 14.3 years.

The diagnoses of the diseases were established in accordance with the latest clinical guidelines. The diagnosis of nIGGG was based on the absence of spontaneous puberty and low levels of sex steroids

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(testosterone <3.4 nmol/L in boys; estradiol <73 pmol/L) in girls against the background of normal or insignificantly low levels of gonadotropins and preserved sense of smell.

The patients were divided into 2 groups:

Group 1 – patients with + diffuse goiter of 1-2 st, with hypothyroidism – 42 patients,

Group 2 – patients with – 48 patients,

The control group consisted of 20 healthy individuals of appropriate middle age (10 boys and 10 girls).

All 90 patients underwent a range of examinations, including the study of endocrine status, general clinical, biochemical, hormonal (GH, LH, FSH, prolactin, TSH, testosterone, cortisol, free thyroxine, etc.) in the laboratory of hormonal research of the RSNMC Endocrinology of the Ministry of Health of the Republic of Uzbekistan. In addition, X-ray (X-ray of the hand and Turkish saddle, CT/MRI of the Turkish saddle and adrenal glands in all patients, ultrasound of the genitals), anthropometric studies (height, weight, height and weight deficit, target height, centile, growth rate, SDS of height and weight, etc.) were performed on the basis of the international Tanner-Weithouse height and weight map, assessment of the stage of sexual development according to Tanner, karyotyping, and other studies.

All genetic studies were carried out by the NDC Immunogen test at the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan on the basis of cooperation agreement No. 10 dated 16.12.21 with the RSNPME of the Ministry of Health of the Republic of Uzbekistan. The material for the study was venous blood samples collected in vacuum tubes with EDTA as an anticoagulant.

Statistical processing of the results was carried out using the standard application package OpenEpi V.9.2.

Research results and discussion. Table 1 shows the distribution of the selected 90 patients by age and stage of puberty.

Table 1.
Distribution of the selected 90 patients by age, sex and
The 5 stages of puberty according to J. Tanner

Age, years,	Total by Chronological Age	Total stages of puberty according to Tanner by XB	Total stages of puberty according to Tanner according to examination data
Boys, n=73			
10 ± 0.5 years	-	I	-
11.7 ± 0.6 years	10 (13,7%)	II	I
13.2 ± 0.8 years	16 (21,9%)	III	I
14.7 ± 0.6 years	22 (30,1%)	IV	II
15.5 ± 0.7 years	25 (34,2%)	V	III
Girls, n=17			
10 ± 0.5 years	-	I	-
11.7 ± 0.6 years	-	II	-
13.2 ± 0.8 years	3 (17.6%)	III	I
14.7 ± 0.6 years	8 (47%)	IV	II
15.5 ± 0.7 years	6 (35.4%)	V	III

As can be seen from Table 1, the most common patients among the examined were at the age of 14.7 years - 22 boys and 8 girls (Tanner's IV by age). At the same time, the stage of puberty at the examination corresponded to II in both 22 boys (30.1 %) and 8 girls (47%).

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In general, when assessing the stage of puberty, it was revealed that 30 adolescents had delayed puberty, that is, puberty corresponded to stage 2 at the age of 13-14 years in both boys and girls.

Next, we calculated the average anthropometric indicators (Table 2).

Table 2.

Mean anthropometric indices of patients by groups

Indicators	**control N=20**	**1 gr N=42**	**2 grams N= 48**
Height, cm	162,6 ± 17,34 160.9 ± 16.5	144,5 ± 19,6* 143,5 ± 17,3*	150,3 ± 22,4* 151,2 ± 20,5*
Weight, kg	51.32 ± 7.30 48.18 ± 7.34	40,9 ± 9,4* 38,3 ± 8,42*	43,5 ± 8,7* 39,6 ± 9,6*
Growth deficit	-	18,5 ± 3,8 17.7± 3.9	12,6 ± 4,6 12.7± 3.4
Underweight	-	12,86 ± 0,9 10,3 ± 3,6	10,5 ± 0,6 9,3 ± 2,4
SDS Growth	6.6 ± 1.2	-1,5	-1,6
SDS Weight	3.5 ± 1.3	-1,0	-1,1
Centile	50	25	50
Average Parental Growth	168,4 ± 4,2	167,5 ± 5,3	169,9 ± 3,5
Projected growth	171,4 ± 4,2	170,5 ± 5,3	174,9 ± 3,5
Middle Age	12,5	9, 6 ± 0, 3	8.90 ± 0.5
Bone age	12,5	5,16 ± 0,1	7,96 ± 0,4
KW/PV	1	0,53 ± 0,02	0.75 ± 0,03

Note: The numerator is boys, the denominator is girls, * is the significance of the differences compared to the control, where * is $p < 0.05$

As can be seen from Table 2, the average height and weight in both groups significantly differed from the data of the control group ($p < 0.05$). At the same time, group 1 of patients with nIGP+ hypothyroidism had the lowest mean values of height and weight compared to group 1 of patients with.

The next step in our research was the study of hormonal disorders (Table 3).

Table 3.

Mean Hormone Values in Study Boys

Hormones	**Control n= 10**	**Boys n= 73**	
STG	3.9±0.2ng/ml	1.3± 0.4	p >0,05
ИФР-1	156.5±9.8 ng/mL	119.8±12.7	p <0,05
LH	5.2±0.3 mIU/L	1.21 ±0.3	p <0,05
FSH	5.3±0.1 mIU/L	1.4± 0.5	p <0,05
TSH	2.5±0.2mIU/L	1, 82±0.7	p >0,05
Prolactin	5.7±0.3 ng/mL	4.4 ±0.8	P >0.05
Testosterone	12.6 ±1.6 nmol/L	3.9± 0.2	p <0,05

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Free			
Cortisol	Normal morning 596.5 ± 11.7 nmol/L	589.25±9.3	p >0,05
St. Thyroxin	15, 8 ± 0.9 pmol/L	15.4± 1.4	p >0,05

P is the significance of the differences compared to the control group (P <0.05). For comparison, the table shows the fluctuations in hormone levels from 11 to 16 years of age in the control group (healthy individuals)

As follows from Table 3, the studied patients had a significant decrease in basal values of LH, FSH (p < 0.05) compared to the control group, as well as significantly low levels of free testosterone (svT) of blood plasma (p < 0.05) against the background of normoprolactinemia. Thus, hypogonadotropic hypogonadism was established in the patients.

Table 4 shows the average values of hormones in the studied girls on the 14th day of the cycle.

Table 4.
Mean Hormone Values in Study Girls on Day 14 of the Cycle

Hormones	**Control n= 10**	**Girls n= 17**	
STG	3.9±0.2ng/ml	1.3± 0.2	p >0,05
ИФР-1	163.5±9.8 ng/mL	112.8±10.2	p <0,05
LH	13.2±2.7 mIU/L	1.4 ±0.1	p <0,05
FSH	10.4±2.5 mIU/L	0.9± 0.03	p <0,05
TSH	2.7±0.7 mIU/L	2, 81±0.8	p >0,05
Prolactin	5.8±0.3 ng/mL	4.6 ±0.9	P >0.05
Estradiol Free	129,1 ±10,6 пг/мл	4.9± 0.2	R <0.05
Cortisol	Normal morning 596.5 ± 11.7 nmol/L	595.25±13.3	p >0,05
St. Thyroxin	16, 5 ± 2.6 pmol/L	14.8± 2.4	p >0,05

p- significance of differences compared to the control group (p <0.05). For comparison, the table shows the fluctuations in hormone levels from 11 to 16 years of age in the control group (healthy individuals)

As follows from Table 4, on the 14th day of the cycle, the studied patients had a significant decrease in basal values of LH, FSH (p < 0.05) compared to the control group, as well as significantly low levels of free testosterone (svT) of blood plasma (p < 0.05) against the background of normoprolactinemia. Thus, hypogonadotropic hypogonadism was established in the patients.

Next, we performed calculations of genetic studies (Table 5).

Table 5
Clinical data of 10 patients examined with GNRH 1 gene polymorphism analysis (rs 6185, rs1812594)

№	Patient, Clinical Status	Diagnosis	karyotype	Deleted Marker	Hormonal examination			
					Testosterone (N 0-38 nmol/L)	FSH (n 9.0-30.0	LH- (n 1.7-8.6 mIU/ml)	Estradiol (normal 131-345 pmol/L

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						mIU/ ml)		
1	A., 14 years 3 months SDS Growth – 1.7 V testes 2 ml penis 2 sm G1		46,XY	rs 6185	0.51	0.68	0.45	-
2	S., 12 years 6 months SDS роста + 1.0 V testes - abs penis 3 sm G1		46 XY	rs 6185	0.7	0.73	0.96	-
3	R., 15 years old p 8,0 sm In testes -2 G1		46 XY	rs 6185	0.44	0,56	0,66	-
4	S., 15 years old P – 8 SM In testes -3 G1		46 XY	rs 6185	0,087	0,44	0,46	-
5	M. 14 y.o. SDS Growth - 0.8		46 XX	rs 6185	0.001	0.36	0.56	133
6	K.14 Years SDS Growth - 1.2		46 XX	rs 6185	0.003	0.38	0452	132
7	S.14 years SDS growth - 1.5 In testes -2 G1		46 XY	rs1812594	0.6	0,40	0,43	-
8	R.14 Years SDS Growth - 1.8		46 XX	rs1812594	0.009	0,36	0,63	123

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9	M.14 Years SDS Growth - 1.6 In testes - 2 G1		46 XY	rs1812594	0.8	0,48	0,56	-
10	S.14 years of SDS growth - 1.3		46 XX	rs1812594	0.006	0,51	0,43	130

The control group consisted of 20 healthy children of the appropriate age (10 boys and 10 girls) with the T/T genotype.

Among the examined individuals, the T/T genotype was detected in 64 (71%) patients, the T/C genotype in 22 (24.4%) and 4 (4.4%) had a C/C genotype mutation. It should be noted that in 5 (6.6%) cases, the rs 6185 polymorphism of the GNRH 1 gene was found.

The rs1812594 polymorphism of the GNRH 1 gene was observed in 4 patients in (4.4%)

Findings. 1. The most common patients among the examined were 14.7 years old - 22 boys and 8 girls (Tanner's IV by age). At the same time, the stage of puberty at the examination corresponded to II in both 22 boys (30.1 %) and 8 girls (47%).

2. In general, when assessing the stage of puberty, it was revealed that 30 adolescents had delayed puberty, that is, puberty corresponded to stage 2 at the age of 13-14 years in both boys and girls. At the same time, group 1 of patients with nIGP+ hypothyroidism had the lowest mean values of height and weight compared to group 1 of patients with.

3. Our results confirm that the GNRH 1 gene polymorphism is the genetic cause of. At the same time, out of 90 patients with clinical and hormonal data of, in 5 (6.6%) cases, the rs6185 polymorphism of the GNRH 1 gene and in (4.4%) - rs1812594 was found.

Keywords: boys, girls, puberty and growth retardation, GNRH1 gene polymorphism

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