Клинико-генетические особенности пациентов с множественным дефицитом гормонов аденогипофиза, обусловленным мутациями в гене *PROP1*: эффективность терапии рекомбинантным гормоном роста

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Обоснование. Одной из наиболее частых причин множественного дефицита гормонов аденогипофиза (МДГА) являются генетические дефекты в гене *PROP1*. Дефицит фактора *PROP1* приводит к недостаточности функции соматотрофов, лактотрофов, тиреотрофов, кортикотрофов и гонадотрофов. В настоящее время появилась возможность проводить крупные популяционные исследования пациентов с генетически обусловленным МДГА, описывать их клинико-генетическую гетерогенность и оценивать эффективность длительной терапии данной группы больных рекомбинантным гормоном роста (рГР).

Цель. Оценить спектр мутаций в гене *PROP1* в российской популяции пациентов с МДГА, частоту и возраст возникновения компонентов гипопитуитаризма, эффективность терапии рГР.

Методы. Проанализированы данные 27 пациентов, наблюдавшихся в Институте детской эндокринологии ФГБУ ЭНЦ в 1978—2016 гг., с диагнозом МДГА и генетически подтвержденными мутациями в гене *PROP1*. МДГА устанавливался на основании лабораторных данных и стимуляционных проб, характеризующих функциональную активность гипофиза. Молекулярно-генетическое исследование проводилось методом высокопроизводительного параллельного секвенирования. Использовалась разработанная в отделении наследственных эндокринопатий ФГБУ ЭНЦ панель праймеров CustomAmpliseq_HP, охватывающая кодирующие области следующих генов: *ARNT2, GH1, GHRH, GHRHR, GHSR, GLI2, HESX1, LHX3, LHX4, OTX2, PAX6, POU1F1, PROP1, SHH, SOX2, SOX3*. Все пациенты получали терапию рГР в ростостимулирующей дозе от момента диагностики СТГ-дефицита до достижения конечного роста. Эффективность терапии оценивалась сравнением конечного достигнутого роста с генетически прогнозируемым.

Результаты. В обследованной когорте пациентов с МДГА, обусловленного мутациями в гене *PROP1*, превалировали несемейные случаи (*n*=23), лишь две пациентки были одноплацентарными сестрами-близнецами, двое других пациентов — родными братом и сестрой. При анализе распределения мутаций в гене *PROP1* выявлена hot-point мутация, c.301_302delAG, отмечавшаяся у 24 пациентов (89%, 95% ДИ 71%; 98%). Мутация в локусе c.150delA встретилась у 11 пациентов (41%, 95% ДИ 22%; 61%). Два пациента имели другие мутации (c.629delC и c.43_49delGGGCGAG). Тотальный СТГ-дефицит выявлен у всех пациентов. Частота вторичного гипотиреоза (ВГТ) у пациентов изученной выборки — 78% (95% ДИ 58%; 91%) на момент диагностики СТГ-дефицита и 100% (95% ДИ 81%; 100%) на момент достижения конечного роста. Частота вторичного гипогонадизма (ВГГ) на момент достижения конечного роста — 100% (95% ДИ 81%; 100%), а частота вторичного гипокортицизма (ВГК) — 41% (95% ДИ 22%; 61%). Нормальный уровень пролактина выявлен у 83% (95% ДИ 65%;94%) пациентов. На момент «закрытия зон роста» пациенты, получавшие терапию рГР в ростостимулирующей дозе, достигли генетически прогнозируемого конечного роста.

Заключение. Наиболее частой мутацией в гене *PROP1*, по нашим данным, является удаление оснований AG в кодоне 101 (c.301_302 delAG) — 89% (95% ДИ 71%; 98). У пациентов с МДГА, обусловленным мутациями в гене *PROP1*, отмечается тотальный СТГ-дефицит и в 100% случаев диагностируется вторичный гипотиреоз и вторичный гипогонадизм. Возможность отсроченной манифестации компонентов гипопитуитаризма требует регулярного скрининга уровня тропных гормонов для своевременного назначения заместительной терапии и предотвращения развития жизнеугрожающих состояний. Терапия рГР высокоэффективна при СТГ-дефиците, обусловленном мутациями в гене *PROP1*, и позволяет пациентам достичь генетически прогнозируемого роста в случае ранней диагностики недостаточности гормона роста.

Ключевые слова: *множественный дефицит гормонов аденогипофиза (МДГА), ген PROP1, СТГ-дефицит, рекомбинантный гормон роста (рГР).*

Clinical and genetic features of patients with multiple anterior pituitary hormone deficiency caused by mutations in the *PROP1* gene; the efficacy of recombinant growth hormone therapy

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Rationale. One of the most common causes of multiple anterior pituitary hormone deficiency (MPHD) is genetic defects in the *PROP1* gene. PROP1 deficiency leads to malfunction of somatotrophs, lactotrophs, thyrotrophs, corticotrophs, and gonadotrophs. Now, there is an opportunity to conduct large-scale population studies of patients with genetic MPHD, describe their clinical and genetic heterogeneity, and evaluate the efficacy of long-term therapy of these patients with a recombinant growth hormone (rGH).

Aim. The study aim was to assess the spectrum of *PROP1* gene mutations in the Russian population of MPHD patients, rate and expected age of hypopituitarism components, and efficacy of rGH therapy.

Material and methods. We analyzed the data of 27 patients diagnosed with MPHD and genetically confirmed mutations in the *PROP1* gene who were treated at the Institute of Pediatric Endocrinology of the Endocrinology Research Center (ERC) in 1978—2016. MPHD was diagnosed based on laboratory data and stimulatory tests characterizing the functional activity of the pituitary gland. The molecular genetic study was performed using high-performance parallel sequencing. We used a custom Am-

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pliseq_HP primer panel developed at the Department of Hereditary Endocrinopathies of the ERC, which included coding regions of the following genes: *ARNT2*, *GH1*, *GHRH*, *GHRHR*, *GHSR*, *GLI2*, *HESX1*, *LHX3*, *LHX4*, *OTX2*, *PAX6*, *POU1F1*, *PROP1*, *SHH*, *SOX2*, and *SOX3*. All patients received rGH therapy at a growth-stimulating dose from the time of GH deficiency diagnosis until final height completion. We evaluated the efficacy of therapy by comparing the achieved final height with the genetically expected one.

Results. Non-familial cases prevailed (N=23) in the study cohort of patients with MPHD caused by mutations in the *PROP1* gene; only two patients were monochorionic twin sisters; the other two patients were siblings. An analysis of the distribution of *PROP1* gene mutations revealed a hot-point mutation c.301_302delAG in 24 patients (89%, 95% CI 71%; 98%). A mutation in the c.150delA locus occurred in 11 patients (41%, 95% CI 22%; 61%). Two patients had other mutations (c.629delC and c.43_49delGGGCGAG). Total GH deficiency was detected in all patients. The rate of secondary hypothyroidism (SHT) in patients of the study sample was 78% (95% CI 58%; 91%) at the time of diagnosis of GH deficiency and 100% (95% CI 81%; 100%) at the time of final height. The rate of secondary hypogonadism (SHG) at the time of final height was 100% (95% CI 81%; 100%), and the rate of secondary hypocorticism (SHC) was 41% (95% CI 22%; 61%). The normal level of prolactin was detected in 83% (95% CI 65%; 94%) of patients. At the time of growth plate closure, patients receiving rGH therapy at the growth-stimulating dose achieved the genetically expected final height.

Conclusion. According to our findings, the most common mutation in the *PROP1* gene is a deletion of AG nucleotides in the 101 codon (c.301_302 delAG), which is found in 89% (95% CI 71%; 98) patients. Patients with MPHD caused by mutations in the *PROP1* gene have total GH deficiency and are diagnosed with secondary hypothyroidism and secondary hypogonadism in 100% of cases. The possibility of delayed manifestation of hypopituitarism components requires regular screening of tropic hormone levels for the timely start of substitution therapy and prevention of life-threatening conditions. rGH therapy is highly effective for GH deficiency caused by *PROP1* gene mutations and allows patients to achieve the genetically expected height in the case of early diagnosis of growth hormone deficiency.

Keywords: *multiple anterior pituitary hormone deficiency (MPHD), gene PROP1, GH deficiency, recombinant growth hormone (rGH).*

MPHD is a rare disease that develops due to impaired pituitary function and is characterized by growth hormone deficiency (GH-deficiency), as well as that of one or more other tropic hormones.

Most of childhood cases are cases of congenital hypopituitarism. The exact causes of disease development remain unclear [1]. Descriptions of familial cases of MPHD served as a basic for searching molecular genetic causes of this condition.

Over the past 20 years, a number of genes have been discovered, whose mutations cause the development of hypopituitarism. These include: *ARNT2, GH1, GHRH, GHRHR, GHSR, GLI2, HESX1, LHX3, LHX4, OTX2, PAX6, POU1F1, PROP1, SHH, SOX2, SOX3* [2].

It is known that mutations in *PROP1* gene are the most common cause of genetically defined MPHD in children. According to the literature, mutations of this gene are found in 50—80% of familial cases of MPHD [3].

In 1996 M. Sornson et al. [4] first discovered the mutations of *PROP1* gene in Ames dwarf mice. The first work on identification of the mutations in human *PROP1* gene was published in 1998 [5]. This gene is mapped on the long arm of the chromosome 5 (5q35) and consists of three exons. *PROP1* gene encodes a protein consisting of 226 amino acids. This protein is necessary for expression of *POU1F1* gene and differentiation of *POU1F1*-dependent cell clones (somatotrophs, lactotrophs and thyrotrophs, gonadotrophs) [2, 3].

The wide range of clinical manifestations of MPHD components in patients with mutations in *PROP1* gene was the reason for its targeted studies. In the literature, there are only few descriptions of patients with mutations in this gene who received regular and long-term therapy with rhGH and achieved final growth [16—18]. The published studies demonstrate high effectiveness of rhGH treatment: all children achieved their genetically predicted growth.

A study of a large group of patients with mutations in *PROP1* gene, who received regular rhGH therapy and reached the final growth, will objectively evaluate the effectiveness of this treatment.

The prevalence of mutations in *PROP1* gene in different populations is also of interest. Currently, at least 24 different mutations of this gene have been identified [26], however, large population studies describing the nature and spectrum of these mutations have not been published. It is only known that the most frequent types of mutations are the deletion of two base pairs in codon 101 (301—302delAG) and the deletion of one base pair in codon 50 (150delA) [19].

Objective — the objective of this study was to assess the spectrum of mutations in *PROP1* gene among the patients in the Russian population, the incidence and the age of onset of MPHD components, and the auxological parameters before and after treatment with rhGH.

Material and methods

Study design

The data of 27 people (48% boys, *n*=13), treated at the Institute of Pediatric Endocrinology of the FGBU ENC in 1978—2016 for MPHD with genetically confirmed mutations in *PROP1* gene were examined. The information on 18 patients was collected retrospectively, and on 9 patients, prospectively (the period of prospective observation was 2 to 5 years).

All patients were born from unrelated healthy parents. In 4 people the disease was of familial nature: two

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patients were one placental twin sisters, and two others were siblings. The familial variants of MPHD were excluded for 23 patients in the group.

Eligibility Criteria

Criteria for inclusion in the study group:

— diagnosis of GH-deficiency confirmed by clonidine and/or insulin probes (maximum stimulated GH level <10 ng/ml);

— mutations in *PROP1* gene confirmed by molecular genetic analysis;

— chronological age <16 years and/or «bone age» <15 years for a group of patients who did not reach the final growth and were continuing rhGH therapy;

— chronological age >16 years and/or «bone age» >15 years for a group of patients who reached the final growth;

— the duration of rhGH therapy of at least 2 years at the time of enrollment.

Exclusion criteria:

The absence of a mutation in *PROP1* gene according to molecular genetic analysis;

Presence of pituitary mass lesions;

Presence of severe concomitant diseases.

Conditions of the study

The data of 27 people living in different cities of the Russian Federation and treated in the Institute of Pediatric Endocrinology of the FGBU ENC in 1978—2016 were analyzed. There was no evaluation of relationship between the patients' residence areas and the spectrum of mutations in *PROP1* gene, the incidence and age of onset of hypopituitarism components, and the effectiveness of rhGH therapy.

Duration of the study

The follow-up period ranged from 2 to 38 years.

Description of medical intervention

After examination conducted in accordance with the National Consensus on diagnosis and treatment of somatotropic insufficiency [4] and diagnosis of «GH-deficiency», the patients received rhGH replacement therapy at a calculated dose of 0.033 mg/kg/day continuously until the final growth was achieved.

Primary endpoint

The final growth of the patients was assessed after reaching the chronological age of >16 years and/or «bone age» of >15 years, as well as at detecting a decrease in the growth rate to less than 2 cm per year while on rhGH therapy.

Subgroup analysis

The patients whose chronological age at the time of the analysis was <16 years and/or «bone age» was <15 years were classified as «continuing rhGH therapy» (n=9) and did not participate in the evaluation of the efficacy of rhGH therapy. The patients whose chronological age at the time of the analysis was >16 years and/or whose «bone age» was >15 years were classified as having «attained final growth» (*n*=18). These patients participated in the evaluation of the effectiveness of rhGH therapy. Both groups were included in assessment of the spectrum of mutations in *PROP1* gene, the incidence and age of onset of MPHD components.

Methods of outcome recording

A significant growth retardation (more than −2 SD) and low growth rates were the reasons for contacting the FGBU ENC. Measurement of body length was carried out with a mechanical growth meter to within 0.1 cm. To estimate the degree of deviation of a patient's growth from the population mean, the standard deviation score (SDS) was calculated using Auxology application (Munich Auxology Project, Kromeyer-Hauschild et al, 2001). Genetically predicted growth and its SDS were also calculated using Auxology application (Munich Auxology Project, Kromeyer—Hauschild et al., 2001).

To assess the somatotropic function of the pituitary gland, challenge tests were performed with clonidine and/or insulin. The total GH-deficiency was diagnosed at the maximum stimulated concentration of growth hormone (GH) <7.0 ng/ml, partial one, at a concentration of 7.0 to 10 ng/ml.

The criterion for diagnosis of secondary hypothyroidism (SHT) was lower level of free T4 (fr.T4) in combination with a normal or moderately elevated baseline level of TSH.

Stimulation test with gonadoliberin (LH-RH) was carried out only for two girls and one boy. «Bone age» at the time of the test was 12 and 12.5 years for the girls and 13 years for the boy. Elevation of LH level of <10 U/l was assessed as secondary hypogonadism (SHG).

For the remaining patients (*n*=15), who achieved chronological age >14 years and had «bone age» characteristic typical for the onset of puberty, the diagnosis of SHG was established based on extremely low baseline LH, FSH, estradiol in girls and testosterone in boys and lack of symptoms sexual maturation. The stage of sexual development was evaluated clinically according to Tanner's classification.

Diagnostic criteria for secondary hypocorticism (SHC) were low baseline level of cortisol (<175 nmol/l in the morning) and low stimulated level of cortisol in insulin hypoglycemia (<540 nmol/l). The test with synthetic ACTH was not performed because this drug is not registered in the territory of the Russian Federation.

Hypoprolactinemia was diagnosed based on low level of prolactin (<90 mU/l).

The Greulich & Pyle method was used to assess the degree of skeleton differentiation («bone age»).

Molecular genetic research was carried out using high-performance parallel sequencing. The Custom Ampliseq HP panel of primers developed in the department

***Table 1*. Characteristics of the group of patients with mutations in *PROP1* gene**

Parameter	All Patients (*n*=27)	Boys (*n*=13)	Girls (*n*=14)	$P_{boys-girls}$, Mann—Whitney test
Age of patients at the time of diagnosis of GH deficiency, years	6.16 (5.32 ; 7.56)	5.72 (4.72; 6.56)	7.49 (5.56; 10.72)	0.16
Bone age at the time of diagnosis of GH deficiency, years	2.0 (2.0; 4.0)	2.5 (2.0; 3.5)	2.0 (2.0; 8.0)	0.96
The maximum stimulated level of GH, ng/ml	0.50 (0.10; 3.30)	0.60 (0.10; 3.10)	0.30 (0.10; 3.30)	0.57
Height of patients at the time of initiation of rhGH therapy, cm	97.90 (90.50; 106.00)	96.70 (90.50; 104.60)	99.65(91.00; 113.75)	0.76
Growth SDS at the time of initiation of rhGH therapy	−3.77 (−4.46; −3.06)	−3.25 (−4.25; −2.99)	−4.06 (−5.49; −3.14)	0.18
Growth rate in the first year of rhGH therapy, cm	14.75 (12.91; 17.01)	13.51 (11.98; 17.01)	14.79(12.91; 17.61)	0.25
Growth rate SDS in the first year of rhGH therapy	10.91 (7.76; 11.58)	11.08 (7.05; 11.56)	8.97 (7.76; 15.36)	0.52

of hereditary endocrinopathies of FGBU ENC (head of the department Dr. A.N. Tyulpakov) which encompasses the coding regions of the following genes: *ARNT2, GH1, GHRH, GHRHR, GHSR, GLI2, HESX1, LHX3, LHX4, OTX2, PAX6, POU1F1, PROP1, SHH, SOX2, SOX3* was used in the study.

Sequencing was performed on a PGM semiconductor sequencer (IonTorrent, «LifeTechnologies», USA). Bioinformatic processing of the results of the sequencing was carried out using TorrentSuite 4.2.1 software module (IonTorrent, «LifeTechnologies», USA) and Annovar software package (version 2014Nov12).

To confirm the changes in *PROP1* gene, sequencing of the respective sites was carried out using specific primers according to Senger.

Eight patients underwent the genetic study at the GeNeSIS DNA analysis laboratory in Leipzig, Germany (GeNeSIS DNA Laboratory, Leipzig University Children's Hospital, Leipzig, Germany, head of the laboratory Professor R. Pfaffle).

Ethical assessment

Ethical assessment was carried out by the local ethical committee of the FGBU «Endocrinology Research Center», Minutes No. 12 of October 22, 2014.

Statistical analysis

Statistical processing of data was carried out using Statistica v.8.0 application software package («StatSoft-Inc.», USA). The median, 1st and 3rd quartiles were used as parameters of distributions of quantitative data. The Mann—Whitney test was used for comparison of independent groups based on the quantitative characteristic, and the Wilcoxon test was used for comparison of dependent groups. The threshold level of statistical significance was 0.05.

The results of checking the statistical hypotheses are given in **Tables 1 and 5**.

The results presented in Table 1 required no correction for PMS, since none of the null hypotheses was rejected.

The results presented in Table 5 were also not corrected for PMS, since 8 out of 10 null hypotheses were not rejected, and the remaining 2 null hypotheses were rejected with a high level of significance, and even the use of Bonferroni's correction would not have led to their acceptance (at an adjusted significance level of 0.05/10 = 0.005).

95% confidence intervals (CI) for relative frequencies were calculated.

Results

Subjects (participants) of the study

The average age of the patients (n=27) at the time of diagnosis of the GH-deficiency was 6.16 years (5.32, 7.56). At the time of data analysis, 18 patients (44% boys, *n*=8) have achieved the final growth, while the remaining children continued their rhGH therapy due to their age and «open growth zones». The duration of rhGH therapy in 18 patients who had reached the final growth was 9.64 years (5.00, 11.16).

The growth of patients at the beginning of rhGH therapy was 97.90 cm (90.50, 106.00) (*n*=27). In all 27 patients, there was a very pronounced degree of stunting: median SDS of growth at the time of diagnosis of GH-deficiency = −3.77 (−4.46; −3.06). A more pronounced growth retardation was observed in girls **(Table 1)** and was associated with somewhat later diagnosis of GH deficiency. However, there was no statistically significant differences in SDS of growth at the time of diagnosis of GH deficiency between boys and girls (*p*=0.18) **(Table 1)**.

All patients were diagnosed with "total GH-deficiency" based on extremely low stimulated level of GH in a test with clonidine (the maximum stimulated level of the GH was 0.50 ng/ml (0.10, 3.30)) (*n*=27). Four patients were additionally tested with insulin. This probe also confirmed total GH deficiency (the maximum concentration of GH was 0.6 to 1.96 ng/ml). Median «bone age» at the time of diagnosis of GH-defi-

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***Table 2*. Results of LH-RG probe in 3 patients with mutations in *PROP1* gene**

	1/F	2/F	3/M
Chronological age, years	16.32	15.64	17.08
«Bone age», years	12.5	12.0	13.0
Baseline level of LH, U/l	0.1	0.1	0.3
Baseline level of FSH, U/l	0.1	0.1	0.1
Maximum stimulated level of LH, U/l	0.1	0.1	0.7
Maximum stimulated level of FSH, U/l	0.1	0.8	1.3
Baseline level of testosterone, nmol/l			0.7
Baseline level of estradiol, pmol/l	20	—	

ciency was 2.0 years (2.0, 4.0). A significantly younger «bone age» compared to the chronological age was noted in all patients **(Table 1)**.

Main outcomes of the study

At the time of diagnosis of GH-deficiency, SHT was diagnosed in 21 patients (78%, 95% CI 58—91). Ten (48%) patients (95% CI 30—66) were diagnosed with SHT concomitantly with GH deficiency, 7 (52%) patients (95% CI 34—70) were diagnosed earlier. The median age at the time of SHT diagnosis was 6.24 years (4.40, 9.32). The level of free T4 was 6.40 pmol/l (4.43, 7.50), baseline TSH, 1.50 mU/l (0.47, 2.26). All patients received levothyroxine therapy with regular dose adjustment. SHT was detected in 100% (95% CI 81—100) of patients who achieved the final growth (n=18).

It was not possible to evaluate the prevalence of SHG at the time of diagnosis of GH deficiency, since all patients were pre-puberty. However, the analysis of the archival data of the patients who achieved the final growth showed that at the end of the observation, all patients were diagnosed with SHG. The age at diagnosis was 16.56 years (14.56, 17.08), «bone age» was 14.0 years in boys (14.0, 14.5) and 13.0 years in girls (13, 0; 14.0). The baseline level of LH was 0.70 U/l (0.2, 1.5), of FSH, 0.90 U/l (0.50, 1.20), testosterone 0.70 nmol/l (0.10, 1.00), estradiol, 21.5 nmol/l (20.00, 23.00).

Two girls, whose «bone age at the time of SHG diagnosis was 12 and 12.5 years, and one boy, whose «bone age» was 13 years, were tested with LH-RH **(Table 2)**.

SHC, which at the time of diagnosis of GH-deficiency was not detected in any patient, was identified in 11 (41%) people (95% CI 25—58) at the time of the analysis of the data. The median age at SHC diagnosis was 9.40 years (9.16, 13.32), baseline level of cortisol was 95.0 nmol/l (82.0, 98.7).

One of the features of MPHD in patients with mutations in *PROP1* gene is prolactin deficiency. This condition has no clinical manifestations in children and adolescents and does not require treatment. In our study, the absolute majority of 15 (83%) patients (95% CI 65—94) had normal baseline prolactin level of 189.50 mU/l (95.60, 250.30) at the time of achieving the final growth. Only 3 (17%) patients (95% CI 17—35) had low baseline level of prolactin.

For the first time, the features of mutations in *PROP1* gene were analyzed in a large group of patients in the Russian population **(Table 3)**. Among 27 patients, there were only 4 familial cases of the disease, whereas most similar studies conducted earlier were based on the analysis of familial cases.

The most common mutation in *PROP1* gene in our study was c.301_302delAG; it was found in homo- or heterozygous state in 24 (89%) patients (95% CI 71—98). The mutation at c.150delA locus was detected in 3 patients in homozygous state and in 8 (41%) patients in heterozygous one (95% CI 22—61), while one patient had other mutations **(Table 3)**.

Additional outcomes of the study

Analysis of the final and genetically predicted growth of the patients demonstrated high efficacy of rhGH therapy in patients with mutations in *PROP1* gene **(Table 4)**. There were no statistically significant differences between the final achieved growth and genetically predicted one in either boys or girls.

Adverse events

The patients were assessed for the following potential adverse events, which could lead to dose reduction or discontinuation of rhGH therapy and termination of participation in the study: edema, arthralgia, benign intracranial hypertension, prepubertal gynecomastia. The absence of adverse events was noted in all patients throughout the observation period.

Discussion

Summary of the primary endpoint

The most common mutation in *PROP1* gene is the deletion of GA pairs in exon 2 (c.301_302delAG). At the time of achieving the final growth 100% patients with mutations in this gene were diagnosed with total GH-deficiency, secondary hypothyroidism and secondary hypogonadism. High probability of development of sec-

***Table 3*. Mutations in *PROP1* gene in 27 patients in the Russian population**

Mutation site	Number of patients
c.301_302delAG p.L102CfsX8/ c.301_302delAG p.L102CfsX8	14
c.150delA p.R43DfsX112/ c.301_302delAG p.L102CfsX8	8
c.150delA p.R43DfsX112/ c.150delA p.R43DfsX112	3
c.629delC p.P210HfsX26/c301_302delAG pL102CfsX8	1
c.43_49delGGGCGAG p.G15SfsX148/c301_302delAG pL102CfsX8	1

***Table 4*. Characteristics of the group of patients with mutations in PROP1 gene who have reached the final growth**

Characteristics	Entire group (n=18)	Boys (n=8)	Girls (n=10)	Pboys—girls, Mann—Whitney test
Final growth of the patients, cm	167.3 (161.0; 175.0)	176.0 (172.0; 181.1)	162.6 (158.8; 167.3)	0.003
Genetically predicted growth, cm	168.5 (163.0; 176.5)	177.5 (174.2; 179.2)	163.0 (162.5; 168.0)	0.001
P, Wilcoxon test	0.80	0.69	0.39	
Final growth of the patients (SDS)	0.24 (–0.41; 0.88)	0.24 (–0.41; 0.98)	0.09 (–0.56; 0.88)	0.88
Genetically predicted growth (SDS)	0.27 (–0.03; 0.72)	0.42 (–0.07; 0.68)	0.16 (0.08; 1.00)	0.978
P, Wilcoxon test	0.60	0.46	0.85	

ondary hypocorticism and subsequence severe life-threatening conditions (acute adrenal insufficiency) requires constant monitoring of the baseline cortisol level and, if necessary, immediate initiation of substitution therapy. Hypoprolactinemia occurred in 25% of the patients in the study and did not require medical treatment. All patients with mutations in gene *PROP1* reached their genetically predicted final growth by the time of «closure of growth zones» (n=18).

Discussion of the primary endpoint

According to international studies, patients with mutations in *PROP1* gene exhibit significant lag in growth, associated with severe intrauterine deficiency of GH and TSH. In our study, at the time of diagnosis of GH-deficiency, all patients had extremely pronounced degree of stunting. There were no statistically significant gender differences in growth SDS. These results are consistent with the data of Russian and foreign colleagues, but it should be noted that the age of diagnosis of GH-deficiency was lower than in the previous studies.

It has been repeatedly shown that patients with mutations in *PROP1* gene have total GH deficiency. However, in 1995 J. Parks and others [9] first raised the issue of possibility of partial GH-deficiency in such patients. The authors described a familial case of MPHD associated with defects in *PROP1* gene, where one of three children with the same mutation had partial GH deficiency. This publication is the only and ambiguous description of partial GH deficiency in a patient with a mutation in *PROP1* gene.

In our work [26], total GH deficiency was detected in all patients. These results are consistent with the latest published data. In a study conducted by F. Pernasetti [11] et al., 10 members of the same family with the 301_302delAG mutation in *PROP1* gene had the maximum stimulated level of GH in the range of 0.1 to 2.1 ng/ml. The age of patients at the time of diagnosis of GH deficiency was 8 to 67 years, and growth SDS at the time of sampling was from –5.2 to –10 SD. O.A. Chikulaeva [20] examined 17 patients aged 3.5 to 15 years with mutations in the gene. The mean age at diagnosis of GH deficiency was 8.61±4.46 years, growth SDS was –1.83 to –6.44. The maximum release of GH in the stimulation probe ranged from 0.1 to 4.2 ng/ml. The total nature of the GH deficiency in case of defects in *PROP1* gene indicates the need for the earliest possible diagnosis and timely rhGH therapy, since delayed diagnosis and lack of therapy can result in pronounced stunting. On the other hand, the heavier the deficiency of endogenous growth hormone is, the more effective the therapy can be (if initiated at an early age): in children with total GH deficiency, even the minimal increase in IGF-1 leads to significant increase in the growth rate, whereas in case of relatively preserved GH secretion the increase in IGF-1 concentration is accompanied by less noticeable changes [13, 14].

The frequency, prevalence and age of onset of other components of MPHD in patients with mutations in *PROP1* gene are actively studied. Since the protein encoded by *PROP1* regulates the development of somatotrophs, lactotrophs, thyrotrophs and gonadotrophs, mutations of this gene can lead to deficit of all the hormones produced by these types of cells.

According to the literature, central hypothyroidism is the first manifestation of hypopituitarism in most patients with mutations in *PROP1* gene, and the age of initiation of rhGH therapy often coincides with the age of

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administration of thyroxine [13, 19, 20]. In our study, SHT was either the first manifestation of the disease, or it was diagnosed simultaneously with GH deficiency. In patients who did not have SHT at the time of diagnosis, central hypothyroidism developed later, and eventually affected 100% of the patients in the study.

Even though PROP1 factor is important for the differentiation of gonadotrophs during fetal development, the incidence of SHG among patients with mutations in *PROP1* gene varies greatly. In addition, the degree of deficiency of gonadotropic hormones can fluctuate from complete absence of puberty to spontaneous development of puberty [6]. The data of our study indicate 100% probability of development of hypogonadism in case of mutations in *PROP1* gene. The stage of puberty at the time of reaching pubertal values of «bone age» in both boys and girls corresponded to «Tanner 1». No evidence of spontaneous onset of puberty has been observed in any patient. After the diagnosis of SHG, all patients were prescribed substitution therapy with sex steroids. At the time of follow-up, none of the patients had any children.

The most frequently discussed issue is the prevalence of SHC among patients with mutations in *PROP1* gene. In a study by F. Pernasetti et al. [13], insulin hypoglycemia test revealed low cortisol levels in 5 of 6 patients over the age of 40 (43, 48, 51, 58 and 67 years) and in only 1 in 4 patients aged 8 to 20 years. All patients had the homozygous 301_302delAG mutation in *PROP1* gene. In 1999, two family members from the Dominican Republic with the 301_302delAG mutation (age of 17 and 40 years old) were described [21], as well as 5 patients with the c.150delA mutation (patients aged 14—68 years) [19] Normal levels of baseline cortisol were observed. However, the authors did not report a dynamic observation of these patients.

In 1998 J. Parks and colleagues [22] reported that only one of the three Polish patients with the 301_302delAG mutation in *PROP1* gene had ACTH deficiency, but ACTH levels and patient age were not reported. In the study by A. Rosenbloom et al. 5 patients with the R120C mutation at the age of 14—29 years had normal baseline and stimulated cortisol values (insulin hypoglycemia test) [14].

In our study, hypocorticism developed in less than 50% the patients. However, it was impossible to predict the probability of its development. Therefore, given the severe and life-threatening manifestations of acute adrenal insufficiency, regular monitoring of the level of cortisol is recommended for the timely administration of replacement therapy with glucocorticoids.

The frequency and degree of hypoprolactinemia in patients with mutations in *PROP1* gene also vary. Histological, immunohistochemical and fluorescent *in situ* hybridization analysis of some pituitary samples of Ames mice revealed low number of cells stained with antibodies to prolactin [6]. The low level of prolactin was detected in all patients observed by A. Rosenbloom: 0.8—3.5 ng/ml (norm 3.6—12.0), whereas F. Pernasetti et al. did not assess the level of prolactin in patients [13 ,14].

According to our study, most patients did not have lower baseline level of prolactin, which, nonetheless, does not preclude the possibility of having decreased stimulated secretion of prolactin. In O.A. Chikulaeva's study, all patients demonstrated "reduced: reaction of prolactin to stimulation with thyrolebirin, despite normal baseline level of prolactin. In contrast to the vitally important screening for hypocorticism, the importance of dynamics assessment of the level of prolactin is controversial, since hypoprolactinemia does not affect the quality of life of patients and does not require medical treatment.

One of the important predictors of the effectiveness of rhGH therapy is the rate of growth in the first year of treatment. During this period, the rate of growth is not only significantly higher than prior to the treatment, but it also outstrips the age norm. According to the literature, during the first year of rhGH therapy, the growth of children with GH deficiency increases by 7.3±2.5 cm [9, 15]. In our study, the rate of growth in the first year of treatment was comparable to that of patients with idiopathic MPHD, the median growth rates in the first year of treatment indicate good response to rhGH therapy.

Evaluation of features of mutations in *PROP1* gene is of particular interest, since the largest group of patients who underwent mutation analysis for this gene included 46 people, 24 of whom had familial cases of MPHD [26]. The results of our study can be considered significant for estimating the true incidence of this particular type of molecular and genetic defect, since we obtained data for one of the largest cohorts of unrelated patients.

The first attempts to estimate the prevalence of localizations of mutation in *PROP1* gene were made back in 1998. In the study of O.V. Fofanova et al. [27] mutations in *PROP-1* gene were detected in 8 patients (57.14%) of a total of 14 patients (28.57% boys, n=4) with MPHD. Homozygous mutation 301_302delAG occurred in 3 (37.50%) patients, heterozygous mutation 301_302delAG/149delGA, in 5 (62.50%) patients in the Russian population.

The second major domestic study is the work of O.A. Chikulaeva [20]. It studied the localization of mutations in *PROP1* gene in 17 Russian patients: 8 patients had a homozygous mutation 301_302delAG; 4 had the compound heterozygote c.150delA / c.301_302delAG; 3, c.301_302delAG/C294T; 1 child, c.150delA and 1 more, C294T/c.150delA.

Among our patients, the most frequent types of mutations were the deletion of two base pairs in codon 101 (301-302delAG) and the deletion of one base pair in codon 50 (150delA), which is consistent with the results of the previous studies [19, 24, 26]. These mutations cause a shift in the reading frame, which in turn leads to the synthesis of a truncated protein (S109X) with impaired function [23, 24]. Since the most frequent changes in patients

are found in the sequence of nucleotides in codons 101 and 50 (these changes occur in 97% of the mutations in *PROP1* gene [19, 24, 26]), the molecular genetic analysis of the causes of hypopituitarism should include the initial screening of the mutations in these codons of *PROP*1 gene.

Limitations of the study

The availability of methods of molecular genetics creates new opportunities for personalized approach to diagnosis, selection of treatment tactics and monitoring of patients with GH deficiency, improving our understanding of etiology and pathogenesis of this condition. Assessment of larger groups of patients with involvement of large medical centers will allow more detailed study of the prevalence of mutations in *PROP1* gene among patients in the Russian population and more objective assessment of the incidence and age of onset of components of the MPHD. Examination of a larger group of patients, who regularly and continuously receive rhGH therapy before achieving the final growth, will allow more objective evaluation of the effectiveness of this therapy.

Conclusion

According to our study, the most common mutation in *PROP1* gene, is the deletion of AG bases in codon 101 (p.301_302 delAG (89%, 95% CI 71—98). In patients with MPHD caused by mutations in *PROP1* gene, total GH deficiency and secondary hypothyroidism and secondary hypogonadism are diagnosed in 100% of cases. Potential delayed manifestation of components of hypopituitarism requires regular screening of tropic hormones level to ensure timely administration of substitution therapy and prevention of development of life-threatening conditions. rhGH therapy is highly effective in case of GH deficiency caused by mutations in *PROP1* gene, and allows patients to achieve genetically predicted growth in case of early diagnosis of growth hormone deficiency.

ADDITIONAL INFORMATION

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ЛИТЕРАТУРА | REFERENCES

1. Дедов И.И., Петеркова В.А., Нагаева Е.В. Гормон роста в современной клинической практике. // Лечащий врач. — 2007. — №2. — С. 13—15. [Dedov II, Peterkova VA, Nagaeva EV. Gormon rosta v sovremennoy klinicheskoy praktike.*Lechashchiy vrach*. 2007;(2):13-15. (шт Russ.)]
2. Pfäffle R, Blum WF. Understanding the Genetics of Growth Hormone Deficiency: A Reference Guide: TMG Healthcare Communications; 2000.
3. Dattani MT. Growth hormone deficiency and combined pituitary hormone deficiency: does the genotype matter? *Clin Endocrinol (Oxf)*. 2005;63(2):121-130. doi: 10.1111/j.1365-2265.2005.02289.x.
4. Петеркова В.А., Фофанова О.В., Тюльпаков А.Н. и др. Диагностика и лечение соматотропной недостаточности у детей. Национальный Консенсус. — М.; 2005. [Peterkova VA, Fofanova OV, Tyulpakov AN, et al. *Diagnostika i lechenie somatotropnoy nedostatochnosti u detey*. National consensus. Mowcow; 2005. (in Russ.)]
5. Sornson MW, Wu W, Dasen JS et al. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature*. 1996;384(6607):327-333. doi: 10.1038/384327a0.
6. Duquesnoy P, Roy A, Dastot F et al. Human Prop-1: cloning, mapping, genomic structure. *FEBS Lett*. 1998;437(3):216-220. doi: 10.1016/s0014-5793(98)01234-4.
7. Parks JS. Heritable Disorders of Pituitary Development. *J Clin Endocrinol Metab*. 1999;84(12):4362-4370. doi: 10.1210/jc.84.12.4362.
8. Phelps CJ, Hurley DL. Pituitary Hormones as Neurotrophic Signals: Update on Hypothalamic Differentiation in Genetic Models of Altered Feedback2. *Proc Soc Exp Biol Med*. 1999;222(1):39-58. doi: 10.1111/j.1525-1373.1999.09994.x.
9. Gage PJ. Ames dwarf mice exhibit somatotrope commitment but lack growth hormone-releasing factor response.*Endocrinology*. 1995;136(3):1161-1167. doi: 10.1210/en.136.3.1161.
10. Parks JS, Tenore A, Bongiovanni AM, Kirkland RT. Familial Hypopituitarism with Large Sella Turcica. *N Engl J Med*. 1978;298(13):698-702. doi: 10.1056/nejm197803302981302.
11. Pernasetti F. Impaired Adrenocorticotropin-Adrenal Axis in Combined Pituitary Hormone Deficiency Caused by a Two-Base Pair Deletion (301-302delAG) in the Prophet of Pit-1 Gene. *J Clin Endocrinol Metab*. 2000;85(1):390-397. doi: 10.1210/jc.85.1.390.
12. Rosenbloom AL. Clinical and Biochemical Phenotype of Familial Anterior Hypopituitarism from Mutation of the PROP1 Gene. *J Clin Endocrinol Metab*. 1999;84(1):50-57. doi: 10.1210/jc.84.1.50.
13. Пятушкина Г.А. Полиморфизм гена рецептора гормона роста и эффективность рекомбинантных препаратов гормона роста. // Вопросы практической педиатрии. — 2007. — Т. 2. — №5. — С. 12—17. [Pyatushkina GA. Polimorfizm gena retseptora gormona rosta i effektivnost' rekombinantnykh preparatov gormona rosta. *Problems of practical pediatrics*. 2007;2(5):12-17. (in Russ.)]
14. Fujieda K, Hanew K, Hirano T et al. Growth response to growth hormone therapy in patients with different degrees of growth hormone deficiency. *Endocr J*. 1996;43 Suppl:S19-25.
15. Hartmann K, Ittner J, Muller-Rossberg E et al. Growth hormone treatment adherence in prepubertal and pubertal children with different growth disorders. *Horm Res Paediatr*. 2013;80(1):1-5. doi: 10.1159/000351800.
16. Rocha MG, Marchisotti FG, Osorio MG et al. High prevalence of pituitary magnetic resonance abnormalities and gene mutations in a cohort of Brazilian children with growth hormone deficiency and response to treatment. *J Pediatr Endocrinol Metab*. 2008;21(7):673-680. doi: 10.1515/jpem.2008.21.7.673
17. Mendonca BB, Osorio MG, Latronico AC et al. Longitudinal hormonal and pituitary imaging changes in two females with combined pituitary hormone deficiency due to deletion of A301,G302 in the PROP1 gene. *J Clin Endocrinol Metab*. 1999;84(3):942-945. doi: 10.1210/jcem.84.3.5537.

18. Lee JK, Zhu YS, Cordero JJ et al. Long-term growth hormone therapy in adulthood results in significant linear growth in siblings with a PROP-1 gene mutation. *J Clin Endocrinol Metab*. 2004;89(10):4850-4856. doi: 10.1210/jc.2003-031816.
19. Deladoey J, Fluck C, Buyukgebiz A et al. «Hot spot» in the PROP1 gene responsible for combined pituitary hormone deficiency. *J Clin Endocrinol Metab*. 1999;84(5):1645-1650. doi: 10.1210/jcem.84.5.5681.
20. Чикулаева О.А. Молекулярно-генетические, гормональные и иммунологические особенности врожденной соматотропной недостаточности у детей: Дисс. ... канд.мед. наук. — М.; 2005. [Chikulaeva OA. Molekulyarno-geneticheskie, gormonal'nye i immunologicheskie osobennosti vrozhdennoy somatotropnoy nedostatochnosti u detey [Dissertation]. Moscow; 2005. (in Russ.)]
21. Mendonca BB, Osorio MG, Latronico AC et al. Longitudinal hormonal and pituitary imaging changes in two females with combined pituitary hormone deficiency due to deletion of A301,G302 in the PROP1 gene. *J Clin Endocrinol Metab*. 1999;84(3):942-945. doi: 10.1210/jcem.84.3.5537.
22. Parks JS, Brown MR, Baumbach L et al. Natural history and molecular mechanisms of hypopituitarism with large sella turcica. In: Book of Abstracts of the 80th Annual Meeting of The Endocrine Society. 1998; 4:471.
23. Cogan JD, WW, Phillips JA et al. The PROP1 2-Base Pair Deletion Is a Common Cause of Combined Pituitary Hormone Deficiency. *J Clin Endocrinol Metab*. 1998;83(9):3346-3349. doi: 10.1210/jc.83.9.3346.
24. Fofanova OV, Takamura N, Kinoshita E et al. A mutational hot spot in the Prop-1 gene in Russian children with combined pituitary hormone deficiency. *Pituitary*. 1998;1(1):45-49.
25. Bottner A, Keller E, Kratzsch J et al. PROP1 mutations cause progressive deterioration of anterior pituitary function including adrenal insufficiency: a longitudinal analysis. *J Clin Endocrinol Metab*. 2004;89(10):5256-5265. doi: 10.1210/jc.2004-0661.
26. Lebl J, Vosahlo J, Pfaeffle RW et al. Auxological and endocrine phenotype in a population-based cohort of patients with PROP1 gene defects. *Eur J Endocrinol*. 2005;153(3):389-396. doi: 10.1530/eje.1.01989.

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