PREVALENCE OF ICHTHYOSIS VULGARIS AND FREQUENCY OF *FLG* R501X AND 2282DEL4 MUTATIONS IN THE POPULATION OF THE ROSTOV REGION

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Ichthyosis vulgaris (IV), a serious skin condition that runs in families, is actively studied worldwide. In this work we aimed to evaluate the prevalence of IV and frequency of two *FLG* mutations R501X and 2282del4 in the population of the Rostov region. Our genetic epidemiology study of hereditary monogenic disorders covered a total of 497,460 residents of 12 districts to identify 230 separate nosological entities. In the course of the analysis, we calculated the prevalence of IV per district and in the entire region and compared our findings with the results of earlier studies. The average prevalence of IV in the Rostov region was 1:5,025, which is consistent with the average prevalence of the disease across Russia (1:5,151). Tselinsky and Millerovsky districts demonstrated the highest prevalence rates (1:1,942 and 1:2,032, respectively). To evaluate the frequency of two *FLG* mutations R501X and 2282del4, we assayed the samples of 58 patients with IV and 127 healthy unelated controls by PCR followed by the restriction fragment length polymorphism analysis. In patients with IV, the frequency of the 2282del4 mutation was 48.28%, which is in line with European figures and also 30 times higher than in the controls (1.58%), suggesting the pathogenicity of the mutation. The R501X mutation was not identified both in patients with IV and healthy controls.

Keywords: ichthyosis vulgaris, mutations R501X and 2282del4, *FLG* gene, prevalence rates, Rostov region

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РАСПРОСТРАНЕННОСТЬ ВУЛЬГАРНОГО ИХТИОЗА И ЧАСТОТА МУТАЦИЙ R501X И 2282DEL4 В ГЕНЕ *FLG* В РОСТОВСКОЙ ОБЛАСТИ

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Вульгарный ихтиоз (ВИ) — серьезное поражение кожного покрова, передающееся по наследству и активно изучающееся специалистами всего мира. Целью работы стала оценка распространенности ВИ и частот мутаций R501X и 2282del4 в гене *FLG* у населения Ростовской области. Проведено генетико-эпидемиологическое исследование широкого круга моногенной наследственной патологии у населения в 12 районах. Изучена частота мутаций R501X и 2282del4 в гене *FLG* у больных ВИ и в контрольной группе (здоровой популяции). Суммарная численность обследованных составила 497 460 чел. Выявлено 230 нозологических форм. Рассчитана распространенность ВИ в каждом районе и по области в целом, проведен сравнительный анализ с ранее обследованными популяциями. Средняя распространенность ВИ в области составила 1:5025 и соответствует среднему значению по России, равному 1:5151. Определено накопление ВИ в Целинском (1:1942) и Миллеровском районах (1:2032). Методом полимеразной цепной реакции (ПЦР) с последующим анализом полиморфизма длин рестрикционных фрагментов изучена частота мутаций R501X и 2282del4 в гене *FLG* у больных ВИ (58 пациентов) и в контрольной группе (127 здоровых неродственных индивидов). Частота мутации 2282del4 в гене *FLG* среди пациентов с ВИ составила 48,28% (соответствует данным по Европе), в контрольной группе 1,58%. Сравнительный анализ частоты мутации 2282del4 в гене *FLG* в двух группах показал, что среди пациентов с ВИ частота мутации (48,28%) 2282del4 в гене *FLG* в 30 раз превышает частоту в контрольной группе (1,58%), что косвенно подтверждает патогенное действие мутации в группе больных ВИ. Мутация R501X не выявлена у больных ВИ и в контрольной группе.

Ключевые слова: вульгарный ихтиоз, мутации R501X и 2282del4, ген *FLG*, генетико-эпидемиологическое исследование, Ростовская область

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Ichthyosis vulgaris (IV, OMIM #146700, also known as ichthyosis simplex) is the most common and relatively mild type of hereditary nonsyndromic ichthyosis. Usually, its clinical symptoms start to show as early as one month after birth and include dry and flaky skin, plate-like or branny greyish scaling especially prominent on the abdomen, chest and extensor surfaces, follicular keratosis and hyperlinear palms and soles [1, 2].

The disease is caused by mutations in the filaggrin gene (*FLG*, OMIM *135940). This gene is part of the epidermal differentiation complex, a gene cluster on the short arm of chromosome 1q21. It consists of three exons and two introns; exon 3 is responsible for protein synthesis [2].

According to the classic research study conducted in Great Britain, the different forms of ichthyosis affect 1 in every 3,665 individuals: ichthyosis vulgaris strikes 1 in 5,300 individuals, X-linked ichthyosis occurs in 1 per 6,190 male population and autosomal recessive forms – in 1 per 300,000 population. However, the actual frequency of the disease was estimated to be even higher: of 6,051 schoolchildren surveyed by the researchers 24 had the autosomal recessive form, which brings the frequency to 1:250 [3].

European DNA studies conducted in patients with IV have revealed that the most common *FLG* mutations associated with the disease are R510X and 2282del4. According to different estimates, their frequencies vary from 30% to 67.3% [4]. In a study conducted in 2006, slight differences were observed in the frequencies of these mutations between the Irish (4.1% and 0.5%, respectively), Scottish (2.1% and 1.2%, respectively) and American (2.4% and 1.1%, respectively) populations [5]. Another study reported a higher frequency of R501X and 2282del4 genetic variants (2.9% and 1.9%, respectively) in Scottish schoolchildren [6]. In Western Austria, though, these two mutations were found to be identically frequent (1.4%) [7]. Perhaps, such discrepancy is to be blamed on the different approaches to patient screening used by the researchers.

Based on molecular genetic screening of patients with IV carried out in South-East Asia a few other *FLG* mutations were described, including 321delA, S2554X, 441delA, 1249insG, 7945delA, Q2147X, E2422X, and R4307X. The frequency of the 3321delA mutation is estimated to be as high as 52.31% in Asian patents with IV and only 4% in healthy controls. Mutations found in the European populations are rare in Asia [8]. So far, allelic heterogeneity associated with various hereditary disorders has been described for many different populations.

In the Russian city of Novosibirsk, the frequency of the *FLG* 2282del4 mutation is 3.8% [9]. In the Republic of Bashkortostan heterozygous carriers of this mutation account for 3.86% of the control group [10]. The frequencies of the R510X and 2282del4 mutations in the Ukrainian population are 2.1% and 1.0%, respectively [11].

To date, ichthyosis vulgaris is believed to be a semi-dominant disease [1, 5]. Smith et al. have shown that in patients with IV heterozygous for either R501X or 2282del4 genetic variants disease manifestations are mild, while patients homozygous for the R501X mutation and compound-heterozygous carriers of R501X/2282del4 develop severe clinical symptoms [5]. Also, a few authors have reported a dosage-dependent effect of *FLG* mutations [6].

The present study aimed to evaluate the prevalence of IV and frequency of the *FLG* R501X and 2282del4 mutations in the population of the Rostov region.

METHODS

Our genetic epidemiology study was carried out in 12 districts of the Rostov region, including Volgodonskoy, Dubovsky, Yegorlyksky, Zimovnikovsky, Krasnosulinsky, Matveevo-Kurganinsky, Millerovsky, Miasnikovsky, Rodionovo-Nesvetaysky, Tarasovsky, Tselinsky, and Tsimliansky. In total, 497,460 individuals were surveyed [12]. In our work, we relied on the guidelines for genetic epidemiology studies conducted over the period between 2000 and 2017 [13, 14]. Those guidelines were developed at the Research Center for Medical Genetics; they have been used as a basis for Russian epidemiologic research studies for over 30 years. The protocol includes a complete health assessment by different specialists, including a geneticist, pediatrician, dermatologist, ophthalmologist, neurologist, psychiatrist, etc. Using this protocol, up to 4,000 or 5,000 separate monogenic nosological entities can be identified in the course of field research.

Prevalence of ichthyosis vulgaris was calculated based on the ratio of the surveyed individuals to the individuals with IV. The obtained results were compared to the data from the genetic epidemiology studies conducted by the Research Center for Medical Genetics [13, 14] because the approaches to data acquisition described in the available literature vary considerably.

A tendency towards increasing incidence of IV in a few districts of the Rostov region was inferred using the F-distribution test ($\alpha < 0.001$) [15].

Table 1. Prevalence of ichthyosis vulgaris in the population of 12 districts of the Rostov region

District	Total size of population	Number of affected individuals	Prevalence
Volgodonskoy	23,542	5	1:4,708
Dubovsky	23,185	4	1:5,796
Yegorlyksky	36,098	13	1:2,777
Zimovnikovsky	38,071	2	1:19,036
Krasnosulinsky	77,847	0	0
Matveevo-Kurgansky	37,600	11	1:3,418
Millerovsky	75,201	37	1:2,032
Miasnikovsky	37,432	5	1:7,486
Rodionovo-Nesvetaisky	30,760	0	0
Tarasovsky	45,575	0	0
Tselinsky	38,830	20	1:1,942
Tsimliansky	33,319	2	1:16,660
Total	497,460	99	1:5,025

Molecular genetic screening of samples for the R501X and 2282del4 mutations in the *FLG* gene was performed in the Laboratory for Genetic Epidemiology, Research Center for Medical Genetics.

To determine the frequencies of 2282del4 and R501X mutations, we collected blood samples from two groups of participants. The first group included 58 affected individuals with IV identified in the course of our epidemiology study conducted in 12 districts of the Rostov region. The second group included 127 unrelated healthy controls who had been living in Millerovsky or Volgodonskoy districts for at least three generations (71 and 56 individuals, respectively). The participants gave written informed consent covering such aspects of the study as voluntary participation, medical examination, blood collection and publication of the results. In the case of underaged children, informed consent was obtained from their parents. A few families insisted that some of their diseased members should not be examined. The study was approved by the Ethics Committee of the Research Center for Medical Genetics (Protocol No. 5 dated December 20, 2010).

Genomic DNA was isolated from peripheral blood leukocytes using the DIAtom DNAPrep100 kit according to the manufacturer's protocol. Screening for c.2282del4 and R501X mutations was done by PCR followed by the analysis of restriction fragment length polymorphisms. Mutation frequencies were compared between healthy and diseased individuals using Fisher's exact test [15].

RESULTS

In the course of our genetic epidemiology study conducted in 12 districts of the Rostov region, we identified 230 separate hereditary monogenic nosological entities. In the present work, we focus on the prevalence and molecular basis of ichthyosis vulgaris, one of ichthyosis form. In total, we have identified 99 patients with IV coming from 49 nuclear families.

IV prevalence was calculated for each district and the Rostov region in general (Table 1). The analysis demonstrates that most patients with IV reside in Millerovsky (37) and Tselinsky (20) districts, while Tarasovsky, Rodionovo-Nesvetaysky, and Krasnosulinsjy districts are free of IV.

IV is the most prevalent in Tselinsky (1:1,942) and Millerovsky (1:2,032) districts. Figures showing IV prevalence in the European part of Russia are provided in Table 2.

Our study shows that prevalence of the disease in the Rostov region corresponds to the average prevalence of IV (1:5,151) in the European part of Russia. The lowest prevalence (1:88,210) was registered in the Bryansk region, while the highest prevalence (1:2,130) was observed in the Republic of Mari El [13, 14]. In our study, a tendency towards increasing incidence was observed in Millerovsky and Tselinsky districts ($\alpha < 0.001$) as compared with average figures on the Rostov region and Russia in general.

Of all surveyed individuals with IV, 58 patients from 38 families agreed to take a DNA test, which confirmed that 28 individuals were heterozygous for the 2282del4 mutation, making 48.28% of all examined unrelated participants with IV. All tests came negative for the R501X mutation. In the group of healthy controls, two (1.58%) were heterozygous for the 2282del4 mutation. Healthy residents of Millerovsky district also tested positive for this mutation, but negative for R501X.

DISCUSSION

In the course of our epidemiology study, we discovered that clinical manifestations of the disease vary markedly from mild to severe both between and within families. All patients started showing symptoms shortly after birth. The symptoms included mucosal dryness, dry scalp and hair, striate patterns on palms and soles, mildly to severely flaky skin, with small or large greyish plate-like scales localized on the abdomen and lower legs. Some patients had scaly skin on the chest and extensor surfaces; follicular keratosis was also observed. Scaling was present on the scalp and along the vermilion zone.

About half of all patients with IV reside in Millerovsky district or were born there (37 patients from 16 families). They come from the Russian families who have been living in the area for at least three generations. We have identified 50 affected individuals in those families (4 died, 7 moved to other areas), which suggests the founder effect. Using the pedigree analysis, we were able to establish kinship between some of the surveyed families. For example, 5 of 16 families (16 patients with IV) living in the khutor of Gray-Voronets share the same ancestor who was born in the late 19th century, in 1896. Their pedigree is presented in Fig. 1.

The analysis demonstrated that the frequency of the *FLG* 2282del4 mutation is lower than in the European population but consistent with the results obtained in Novosibirsk [7, 9].

Table 2. Prevalence of ichthyosis vulgaris in the surveyed regions of the European part of Russia

Regions of the Russian Federation	Total size of population	Prevalence
Rostov region	497,460	1:5,025
Arkhangelsk region	40,000	1:6,667
Bryansk region	88,210	1:88,210
Kirov region	286,616	1:3,675
Kostroma region	444,476	1:8,386
Tver region	76,000	1:12,667
Krasnodar region	426,700	1:8,534
Republic of Adygea	101,800	1:5,358
Republic of Bashkortostan	250,000	1:6,944
Republic of Mari El	276,900	1:2,130
Republic of Tatarstan	268,894	1:7,469
Udmurt Republic	267,655	1:6,225
Chuvash Republic	264,490	1:3,149
Russia (average)	2791,741	1:5,151

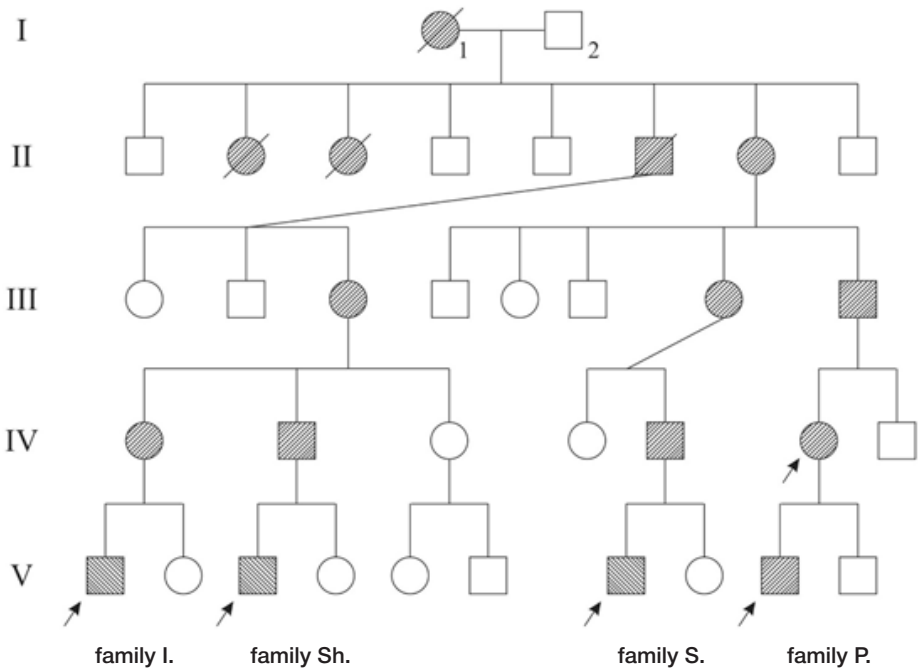

Fig. 1. Pedigree of the family with ichthyosis vulgaris

Our study has revealed significant differences (Fisher's $p < 0.00001$) in the frequencies of *FLG* 2282del4 between individuals with IV (0.483, 28/58) and healthy controls (0.016, 2/127).

CONCLUSIONS

On average, ichthyosis vulgaris occurs in 1 of 5,025 surveyed residents of the Rostov region. Its prevalence varies from 0 in Tarasovsky, Rodionovo-Nesvetaysky and Krasnosulinsky districts to 1:1,942 in Tselinsky and 1:2,032 in Millerovsky districts. The prevalence of the disease in the Rostov region is consistent with the average figures across Russia (1:5,151) [13, 14]. The founder effect is observed for the majority of the surveyed families. Among patients with IV the 2282del4 mutation is 30 times more frequent (48.28%) than among the controls (1.58%), which suggests its pathogenicity. In our study, the R501X mutation was absent in both diseased individuals and healthy controls.

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