The Unique Spectrum of Mutations in Patients with Hereditary Tyrosinemia Type 1 in Different Regions of the Russian Federation

G. V. Baydakova · T. A. Ivanova · S. V. Mikhaylova ·
D. Kh. Saydaeva · L. L. Dzhudinova ·
A. I. Akhlakova · A. I. Gamzatova · I. O. Bychkov ·
E. Yu. Zakharova

Received: 29 June 2018 / Revised: 29 August 2018 / Accepted: 17 September 2018 / Published online: 11 November 2018
© Society for the Study of Inborn Errors of Metabolism (SSIEM) 2018

Abstract *Background:* Hereditary tyrosinemia (HT1) is an autosomal recessive disorder characterized by impaired tyrosine catabolism because of fumarylacetoacetate hydrolyase deficiency. HT1 is caused by homozygous or compound heterozygous mutations in the *FAH* gene. The HT1 frequency worldwide is 1:100,000–1:120,000 live births. The frequency of HT1 in the Russian Federation is unknown.

Aim: To estimate the spectrum of mutations in HT1 in several ethnic groups of the Russian Federation.

Materials and methods: From 2004 to 2017, 43 patients were diagnosed with HT1. The analysis of amino acids and succinylacetone was performed using NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit and a Sciex QTrap 3200 quadrupole tandem mass spectrometer. Bi-directional DNA sequence analysis was performed on PCR products using an ABI Prism 3500.

Results: In the Russian Federation, the most common mutation associated with HT1 (32.5% of all mutant alleles) is c.1025C>T (p.Pro342Leu), which is typical for the Chechen ethnic group. Patients of the Yakut, the Buryat, and the Nenets origins had a homozygous mutation c.1090G>C (p.Glu364Gln). High frequency of these ethnicity-specific mutations is most likely due to the founder effect. In patients from Central Russia, the splicing site mutations c.554-1G>T and c.1062+5G>A were the most prevalent, which is similar to the data obtained in the Eastern and Central Europe countries.

Conclusion: There are ethnic specificities in the spectrum of mutations in the *FAH* gene in HT1. The Chechen Republic has one of the highest prevalence of HT1 in the world.

Introduction

Tyrosinemia type I (hepatorenal tyrosinemia (HT1) OMIM 276700) is an inherited disorder caused by deficiency of fumarylacetoacetate hydrolase (*FAH*, MIM 613871) which is the last enzyme of the tyrosine catabolic pathway. Enzyme deficiency leads to accumulation of toxic metabolites (succinylacetone and fumarylacetoacetate) that influence hepatocytes and cells of the proximal renal tubules, resulting in liver damage and disruption of tubular reabsorption of phosphates (Endo and Sun 2002; Novikov 2012).

The fumarylacetoacetate hydrolase gene (*FAH*) is located on chromosome 15 (locus 15q23-q25). To date, approximately 100 mutations have been found, and most of them are missense mutations (Bergeron et al. 2001).

Communicated by: Piero Rinaldo, MD, PhD

G. V. Baydakova · T. A. Ivanova (✉) · I. O. Bychkov ·
E. Yu. Zakharova

Federal State Budgetary Scientific Institution “Research Center for
Medical Genetics”, Moscow, Russia
e-mail: breaking18@gmail.com

S. V. Mikhaylova
Russian Children’s Clinical Hospital of the Ministry of Health of the
Russian Federation, Moscow, Russia

D. Kh. Saydaeva · L. L. Dzhudinova
Perinatal Center, State Budgetary Institution Maternity Hospital,
Grozny, The Chechen Republic, Russia

A. I. Akhlakova · A. I. Gamzatova
Republican Center for Medical Genetics, Makhachkala, The Republic
of Dagestan, Russia

HT1 is characterized by progressive liver disease and secondary renal tubular dysfunction leading to hypophosphatemic rickets. Two clinical forms of HT1 have been described: the acute form, which presents itself in the first months of life and is associated with acute liver failure, and the chronic form, which appears after the first year of age and leads to the development of hepatic failure or liver tumors (van Spronsen et al. 1994). The main biomarker used to detect the diagnosis of HT1 was an increase in succinylacetone (SA) in blood and urine.

Nitisinone (Orfadin[®]), also known as NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione), blocks the second step in the tyrosine degradation pathway and is successfully used for treatment of HT1. Therefore, early diagnosis of the disorder plays a crucial role in successful treatment (McKiernan 2006; Polyakova 2012).

Newborn screening for HT1 has been introduced in some countries and is based on detection of SA in the blood (Allard et al. 2004).

The aim of the study was to estimate the spectrum of mutations in HT1 and their frequencies in several ethnic groups belonging to different geographical regions of the Russian Federation.

Materials and Methods

Patients

From 2004 to 2017, 43 patients were diagnosed with HT1. The ethnic composition of the patients was as follows: 24 Russians, 13 Chechens, 1 Ingush, 2 Armenians, 1 Yakut, 1 Buryat, and 1 Nenets.

Metabolites

The analysis of amino acids, acylcarnitines, and succinylacetone was performed using NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit (Perkin Elmer Life and Analytical Sciences, Wallac OY, Finland) and a Sciex QTrap 3200 (Sciex, USA) quadrupole tandem mass spectrometer operating based on the positive electrospray ionization technique. The concentrations of amino acids, acylcarnitines, and succinylacetone were calculated automatically using the internal standards and ChemoView software (Sciex, USA).

Molecular Genetics

Amplification of all the coding exons and their flanking regions *FAH* gene was performed by PCR on patient genomic DNA. Bi-directional DNA sequence analysis was

performed on PCR products using an ABI Prism 3500 (Applied Biosystems). Primer sequences and protocol are available upon request.

To evaluate the frequency of the c.1025C>T (p.Pro342Leu) mutation, DNA extracted from dried blood spots of newborns from the Chechen Republic ($n = 2,215$) and the Republic of Dagestan ($n = 201$) were analyzed. Blood specimens (unidentified) were provided by the neonatal screening centers in Grozny and Makhachkala. The specimens were collected during 2016. PCR-RFLP analysis using *MspI* (C[^]CGG) restriction endonuclease (SibEnzyme, Russia) was developed for detection of the c.1025C>T (p.Pro342Leu) mutation. The Hardy-Weinberg equilibrium was used to calculate the frequency of the disorder: $p^2 + 2pq + q^2 = 1$. The confidence interval for frequencies was calculated by the Wilson method.

Results

In 43 patients from the Russian Federation, the diagnosis was made based on typical clinical presentation (hepatic impairment, rickets-like changes) as well as detection of specific HT1-related biomarkers, such as elevated blood and/or urine concentration of succinylacetone, tyrosine, and methionine. Biomarker concentrations in HT1 patients are presented in Table 1. In all patients, genetic testing was conducted to detect *FAH* gene mutations.

In our cohort of patients from different regions of the Russian Federation, 16 different mutations in different combinations were revealed; the most common mutations were c.554-1G>T, c.1062+5G>A, c.1025C>T (p.Pro342Leu), and c.1090G>C (p.Glu364Gln). Clear ethnicity-related peculiarities were observed (Tables 2 and 3). The most frequent mutation in our cohort was c.1025C>T (p.Pro342Leu) (32.5% of mutant alleles).

Table 1 Biochemical changes observed in patients with tyrosinemia type 1

	Control ^a	Acute type ^a	Chronic type ^a
Tyrosine (blood), $\mu\text{M/L}$	81.7 ± 38.8 (3.1–322)	442 ± 136 (352.8–767)	327.1 ± 88.7 (234–618)
Methionine (blood), $\mu\text{M/L}$	26.8 ± 13.4 (3–198)	515.8 ± 318.8 (18.2–1,157)	45.1 ± 44.1 (11.3–171)
Succinylacetone (blood), $\mu\text{M/L}$	0.74 ± 0.3 (0.13–1.9)	32.1 ± 36.2 (5.27–85.2)	9.1 ± 5.9 (2.4–17.5)
Succinylacetone (urine), mM/M creatinine	Undetectable <2	167.3 ± 75.8 (75.4–283)	57.2 ± 33.3 (14–112)

^a Mean $\pm$ deviation (minimum–maximum)

Table 2 Frequencies of the *FAH* gene mutations among patients with tyrosinemia type 1 from the Russian Federation

Mutation	Number of alleles	Frequency (%)
c.1025C>T (p.Pro342Leu)	28	32.5
c.554-1G>T (IVS6-1G>T)	20	23.3
c.1062+5G>A (IVS12+5G>A)	8	9.3
c.1090G>C (p.Glu364Gln)	8	9.3
c.192G>T (p.Gln64His)	4	4.6
c.520C>T (p.Arg174Term)	2	2.3
c.782C>T (p.Pro261Leu)	2	2.3
c.497T>G (p.Val166Gly)	2	2.3
c.698A>T (p.Asp233Val)	1	1.2
c.2T>A (p.Met1Lys)	1	1.2
c.1056C>A (p.Ser352Arg)	1	1.2
c.455G>A (p.Trp152Term)	1	1.2
Variant of uncertain significance	8	9.3

Table 3 Frequencies of some mutations in the *FAH* gene in different ethnic groups

Ethnic group	Frequency of mutations (number of alleles)			
	c.554-1G>T	c.1062+5G>A	c.1025C>T (p.Pro342Leu)	c.1090G>C (p.Glu364Gln)
Russians (n = 24)	41.7 (20)	16.7 (8)	0	0
Chechens (n = 13)	0	0	100 (26)	0
Armenians (n = 2)	0	0	0	0
Yakuts (n = 1)	0	0	0	100 (2)
Buryats (n = 1)	0	0	0	100 (2)
Nenets (n = 1)	0	0	0	100 (2)

Among 24 patients of Russian ethnic group, 20 alleles were identified c.554-1G>T, which is 41.7%; and 8 alleles were identified c.1062+5G>A, which is 16.7% of the sample. 26 alleles c.1025C>T were found in 13 patients of the Chechen Republic, which is 100% of the sample. Yakuts, Buryats, and Nenets were found the same mutation in the homozygous state

Discussion

HT1 is a rare autosomal recessive genetic disorder. The HT1 worldwide frequency is approximately 1:100,000–1:120,000 live births (Mitchell et al. 2001). In some areas, the incidence of HT1 is noticeably higher. In Norway, Finland, and Tunisia, the frequency of HT1 is 1:74,800, 1:60,000, and

1:14,804, respectively (Bliksrud et al. 2012; St-Louis et al. 1994; Nasrallah et al. 2015). The highest prevalence of the disorder is observed in Canada (the Province of Quebec) near Saguenay-Lac Saint-Jean at 1:1,846 live births. Newborn screening has been conducted in the Province of Quebec since 1970. The most predominant mutation in this region is c.1062+5G>A (IVS12+5G>A), accounting for ~90% of all the disease-causing alleles. The HT1 carrier frequency in Quebec is 1:66, while in the region of Saguenay-Lac Saint-Jean, it is estimated to be 1:16–1:20 (De Braekeeler and Larochelle 1990).

In addition to the differences in incidence of the disorder, the distribution of mutations among ethnic groups has also been described. The most common mutation in patients from Northern Europe, Pakistan, Turkey, and the United States is a c.1062+5G>A (IVS12+5G>A) splicing mutation. Another splicing site mutation, c.554-1G>T, is prevalent in Central and Eastern Europe, and in Spain this mutation accounts for approximately 70% of all mutant alleles (Bergman et al. 1998). Some populations demonstrate predominance of certain unique mutations due to the founder effect. In the Finnish population, c.786G>A (p.Trp262X) represents ~88% of all reported HT1 alleles, and the major mutation in Ashkenazi Jews is c.782C>T (p.Pro261Leu) (St-Louis et al. 1994; Elpeleg et al. 2002). All patients from the Province of Quebec are homozygous for the splicing site mutation c.1062+5G>A (Elpeleg et al. 2002). In 19 HT1 patients in Norway, three new small deletions were found: c.615delT (p.Phe205LeufsX2), c.744delG (p.Pro249HisfsX55), and c.835delC (p.Gln279ArgfsX25) in 13.5%, 3.8%, and 1.9% of the alleles, respectively.

In our patients from Central Russia, the splicing site mutations c.554-1G>T and c.1062+5G>A were the most prevalent, which is similar to the data obtained in the Eastern and Central Europe countries (Table 3).

Clear ethnicity-related peculiarities were observed (Tables 2 and 3). The most frequent mutation in our cohort was c.1025C>T (p.Pro342Leu) (32.5% of mutant alleles). This homozygous mutation was identified in 14 patients, 13 of whom were ethnic Chechens (12 from the Chechen Republic and 1 from the Republic of Dagestan) and 1 was Ingush. This mutation has been previously described in the literature and in the Human Gene Mutation Database in a patient from Norway (HGMD CM940753) (heterozygous mutation). A functional analysis using fibroblast culture demonstrated a decrease in enzyme activity associated with this mutation (Rootwelt et al. 1994). Assessment of the mutation using standard software (PolyPhen-2, PROVEAN +SIFT, MutationTaster) shows highly probable pathogenicity of this substitution. According to ExAc, the frequency of this allele is 0.0000247/3, which also confirms its pathogenicity.

Based on the obtained results, it was suggested that the HT1 prevalence is higher in the Chechen Republic compared to other regions of the Russian Federation. The presence of the major mutation allows for analysis of the carrier frequency. 2,215 blood specimens collected from newborns in the Chechen Republic and 201 blood specimens collected from newborns in the Republic of Dagestan were analyzed. Thirty-five carriers of mutation c.1025C>T (p.Pro342Leu) were revealed in the Chechen population examined; no such mutation was found in the blood specimens obtained from newborns in the Republic of Dagestan. Thus, the frequency of the mutant allele p.Pro342Leu in the Chechen Republic was 0.0079 (95% confidence interval, 0.0045998 ± 0.01207), and the estimated frequency of HT1 (q^2) was 0.00006242 (95% confidence interval, $0.00002116 \pm 0.00014568$) or 1:16,020 (95% confidence interval, $1:6,864 \pm 1:47,263$). This frequency is one of the highest in the world. Since the Chechen population consists of local clans (taips), it is necessary to determine the taips of patients from the Chechen Republic to estimate the reason of prevalence of this mutation in the respective subpopulations (and to determine whether it is the result of the founder effect). Since the populations of the Chechens and the Ingush are genetically quite similar (Dibirova et al. 2010), it is reasonable to evaluate the frequency of the p.Pro342Leu mutation in the Ingush ethnic group.

Another interesting finding is the detection of a homozygous c.1090G>C (p.Glu364Gln) mutation in three patients (who were from the Yakut, Buryat, and Nenets ethnic groups). Maksimova et al. found that the carrier frequency of this heterozygous mutation among the Yakuts was 1%. These results suggest that the prevalence of HT1 in this region of the Russian Federation is also quite high and may be up to 1:10,000 live births (Maksimova et al. 2016).

In seven patients, undescribed variants in the heterozygous condition were identified (c.767C>A (p.Ala203Asp), c.772T>C (p.Phe205Ser), c.702G>T (p.Trp234Cys), c.803C>A (p.Ala268Asp), c.998A>C (p.His333Pro), c.706+3G>A (ivs8+3G>A), c.455+1G>A (ivs5+1G>A)). Diagnosis of HT1 was confirmed by biochemical methods: succinylacetone in the urine ranged from 47 to 146 mM/M creatinine and in the blood ranged from 4 to 8 μ M/L and tyrosine in the blood ranged from 113 to 618 μ M/L. Functional diagnosis of confirmation of pathogenicity of the variants was not carried out. But assessment of the variants using standard software (PolyPhen-2, PROVEAN+SIFT, MutationTaster) shows highly probable pathogenicity of these substitutions. It should be noted that the variant c.772T>C (p.Phe205Ser) met twice by patients of the Chuvash Republic.

Common pseudodeficiency allele c.1021C>T (p.Arg341Trp) was not detected in patients with HT1. Frequency of this allele was about 3% in the control sample.

Conclusion

Evaluation of the spectrum of mutations in the *FAH* gene in hereditary tyrosinemia type 1 (HT1) in different populations of the Russian Federation demonstrated ethnic specificity of some mutations. The mutation c.1025C>T (Pro342Leu), which is common for the Chechens, was found to be the most common (32.5%) among mutations leading to this disorder. The estimated prevalence of HT1 in this population, 1:16,020, is one of the highest in the world.

We identify mutation c.1090G>C (p.Glu364Gln), in HT1 patients representing the nations of the peoples of Northern-Eastern Russia, including the Yakuts, the Buryats, and the Nenets. The estimated prevalence of HT1 in the Yakuts may be 1:10,000 live births, which is also quite high.

The obtained data indicate the need for introduction of HT1 newborn screening to the National program in the Chechen Republic and probably in Yakutia.

The authors declare that there is no conflict of interest.

Compliance with Ethics Guidelines

Galina Baydakova, Tatiana Ivanova, Svetlana Mikhaylova, Djamila Saydaeva, Laura Dzhudinova, Ayashat Akhlakova, Amina Gamzatova, Igor Bychkov, and Ekaterina Zakharova declare that they have no conflict of interest.

This study was partially granted by SOBI.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Baydakova G. V. performed the analysis of amino acids and succinylacetone in spots of dried blood, conducted DNA diagnostics to detect mutations in the *FAH* gene, calculated the frequency of the disorder, and calculated the confidence interval for frequencies.

Ivanova T. A. performed the analysis of amino acids and succinylacetone in spots of dried blood and conducted DNA diagnostics to detect mutations in the *FAH* gene.

Mikhaylova S. V. carried out a sample of patients on the territory of the Russian Federation and selection and search of articles and literature.

Saydaeva D. Kh. and Dzhudinova L. L. carried out a sample of patients on the territory of the Chechen Republic and selection and search of articles and literature.

Akhlakova A. I. and Gamzatova A. I. carried out a sample of patients on the territory of the Republic of Dagestan and selection and search of articles and literature.

Bychkov I. O. conducted DNA diagnostics to detect mutations in the FAH gene.

Zakharova E. Yu is the guarantor of the research.

Competing Interests

None declared.

References

- Allard P, Grenier A, Korson MS, Zytkevich TH (2004) Newborn screening for hepatorenal tyrosinemia by tandem mass spectrometry: analysis of succinylacetone extracted from dried blood spots. *Clin Biochem* 37(11):1010–1015
- Bergeron A, D'Astous M, Timm DE, Tanguay RM (2001) Structural and functional analysis of missense mutations in fumarylacetoacetate hydrolase, the gene deficient in hereditary tyrosinemia type I. *J Biol Chem* 276:15225–15231
- Bergman AJ, van den Berg IE, Brink W et al (1998) Spectrum of mutations in the fumarylacetoacetate hydrolase gene of tyrosinemia type I patients in northwestern Europe and Mediterranean countries. *Hum Mutat* 12(1):19–26
- Blikrud YT, Brodtkorb E, Backe PH et al (2012) Hereditary tyrosinaemia type I in Norway: incidence and three novel small deletions in the fumarylacetoacetase gene. *Scand J Clin Lab Invest* 72(5):369–373
- De Braekeeler M, Larochelle J (1990) Genetic epidemiology of hereditary tyrosinemia in Quebec and the Saguenay-Lac-St-Jean. *Am J Hum Genet* 47(2):302–307
- Dibirova KD, Balanovskaya EV, Kuznetzova MA et al (2010) Genetic topography of the Caucasus: four linguistic-geographical regions as indicated by data on chromosome Y polymorphism. *Med Genet* 10:10–14 (in Russian)
- Elpeleg ON, Shaag A, Holme E et al (2002) Mutation analysis of the FAH gene in Israeli patients with tyrosinemia type I. *Hum Mutat* 19(1):80–81
- Endo F, Sun MS (2002) Tyrosinaemia type I and apoptosis of hepatocytes and renal tubular cells. *J Inher Metab Dis* 25:227–234
- Maksimova NR, Gurinova EE, Sukhomyasova AL et al (2016) A novel homozygous mutation causing hereditary tyrosinemia type I in Yakut patient in Russia: case report. *Wiad Lek* 69(2 Pt 2):295–298
- McKiernan PJ (2006) Nitisinone in the treatment of hereditary tyrosinaemia type I. *Drugs* 66(6):743–750
- Mitchell GA, Grompe M, Lambert M, Tanguay RM (2001) Hyper-tyrosinemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The metabolic and molecular bases of inherited disease*. McGraw Hill, New York, pp 1777–1806
- Nasrallah F, Hammami MB, Ben Rhouma H et al (2015) Clinical and biochemical profile of tyrosinemia type I in Tunisia. *Clin Lab* 61(5–6):487–492
- Novikov PV (2012) Tyrosinemia type I: clinical signs, diagnosis and treatment. *Russ Bull Perinatol Pediatr* S3:1–27 (in Russian)
- Polyakova SI (2012) Effectiveness of nitisinone therapy for hereditary tyrosinemia type I. *Russ Pediatr J* 6:59–60 (in Russian)
- Rootwelt H, Chou J, Gahl WA, Berger R, Coşkun T, Brodtkorb E, Kvittingen EA (1994) Two missense mutations causing tyrosinemia type I with presence and absence of immunoreactive fumarylacetoacetase. *Hum Genet* 93(6):615–619
- St-Louis M, Leclerc B, Laine J et al (1994) Identification of a stop mutation in five Finnish patients suffering from hereditary tyrosinemia type I. *Hum Mol Genet* 3(1):69–72
- van Spronsen FJ, Thomasse Y, Smit GP, Leonard JV et al (1994) Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. *Hepatology* 20:1187–1191