

## CARRIER FREQUENCY OF *GJB2* AND *GALT* MUTATIONS ASSOCIATED WITH SENSORINEURAL HEARING LOSS AND GALACTOSEMIA IN THE RUSSIAN POPULATION

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This article continues a series of works estimating carrier frequencies of mutations associated with the development of common monogenic disorders in the Russian population. The study aimed to establish the frequency of *GJB2* and *GALT* mutations in first-time blood donors. Genotyping of 1000 first-time blood donors who identify themselves as Russians and permanently reside in the Russian Federation detected 37 carriers of *GJB2* mutations associated with sensorineural hearing loss (carrier frequency in the sample was 3.7 %, or 1 : 27) and 6 carriers of *GALT* mutations associated with galactosemia (carrier frequency in the sample was 0.6 %, or 1 : 167). In one carrier, concurrent mutations were detected; thus, in total 42 carriers of *GJB2* and *GALT* mutations were detected (carrier frequency in the sample was 4.2 %, or 1 : 24).

**Keywords:** sensorineural hearing loss, *GJB2*, galactosemia, *GALT*, genotyping, Russian population

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Received: 06.12.2016 Accepted: 12.12.2016

## ЧАСТОТА НОСИТЕЛЬСТВА В РОССИЙСКОЙ ПОПУЛЯЦИИ МУТАЦИЙ В ГЕНАХ *GJB2* И *GALT*, АССОЦИИРОВАННЫХ С РАЗВИТИЕМ НЕЙРОСЕНСОРНОЙ ТУГОУХОСТИ И ГАЛАКТОЗЕМИИ

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Статья продолжает цикл работ, посвященных определению частоты носительства в российской популяции мутаций, ассоциированных с развитием распространенных моногенных заболеваний. Целью исследования было установление частоты распространенных в российской популяции мутаций в генах *GJB2* и *GALT* у доноров первичной кроводачи. При генотипировании 1000 доноров первичной кроводачи, идентифицирующих себя как русских и постоянно проживающих на территории Российской Федерации, обнаружены 37 носителей мутаций в гене *GJB2*, ассоциированных с развитием нейросенсорной тугоухости (частота в выборке составила 3,7 %, или 1 : 27), и 6 носителей мутаций в гене *GALT*, ассоциированных с развитием галактоземии (частота в выборке — 0,6 %, или 1 : 167). Выявлен 1 случай сочетанного носительства мутаций, и, таким образом, всего обнаружены 42 носителя мутаций в генах *GJB2* и *GALT* (частота в выборке — 4,2 %, или 1 : 24).

**Ключевые слова:** тугоухость, *GJB2*, галактоземия, *GALT*, генотипирование, российская популяция

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Статья получена: 06.12.2016 Статья принята в печать: 12.12.2016

Nonsyndromic sensorineural hearing loss is an inherited condition (OMIM #220290) characterized by congenital hearing impairment. The most common type of genetic hearing disorders found in developed nations is autosomal recessive nonsyndromic hearing loss associated with a mutation in the *GJB* gene that encodes a protein known as connexin 26. So far, over 90 *GJB2* mutations have been associated with deafness, 35delG mutation being highly prevalent in European and Russian populations. This mutation results in a premature stop codon. The carrier frequency of *GJB2*:35delG can vary

from 1 : 100 to 1 : 30 in European populations and from 1 : 50 to 1 : 25 in some Russian populations [1–5].

Galactosemia is a hereditary disease caused by the deficiency of galactose-metabolizing enzymes. Galactose enters the body in food as a component of disaccharide lactose (milk sugar). It is believed that accumulation of toxic amounts of galactose-1-phosphate in the cells affects cell metabolism and leads to pathology. The most marked changes occur in the liver, kidneys, eye lens and brain. Patients who receive no treatment die within the first months of life from

sepsis or liver failure; all patients develop mental deficiency with typical speech impairment (cluttering). But if a diet is prescribed timely, a child may still develop normally. The disease is linked to the reduced activity of the galactose-1-phosphate uridyl transferase enzyme associated with *GALT* mutations. In healthy individuals, this enzyme catalyzes production of glucose-1-phosphate and uridyl diphosphate-galactose from galactose-1-phosphate and uridyl diphosphate- glucose. Galactosemia follows an autosomal recessive pattern of inheritance; its incidence in the Russian population is 1 in 20 000 births. The most critical mutations for the Russian population are Q188R, K285N, M142K, L358P, IVS3-2A>C [6, 7].

The aim of this study was to determine the frequency of *GJB2* and *GALT* mutations in first-time blood donors who identify themselves as Russians and permanently reside in the Russian Federation.

METHODS

Peripheral blood was collected from 1000 healthy first-time blood donors who identified themselves as Russians.

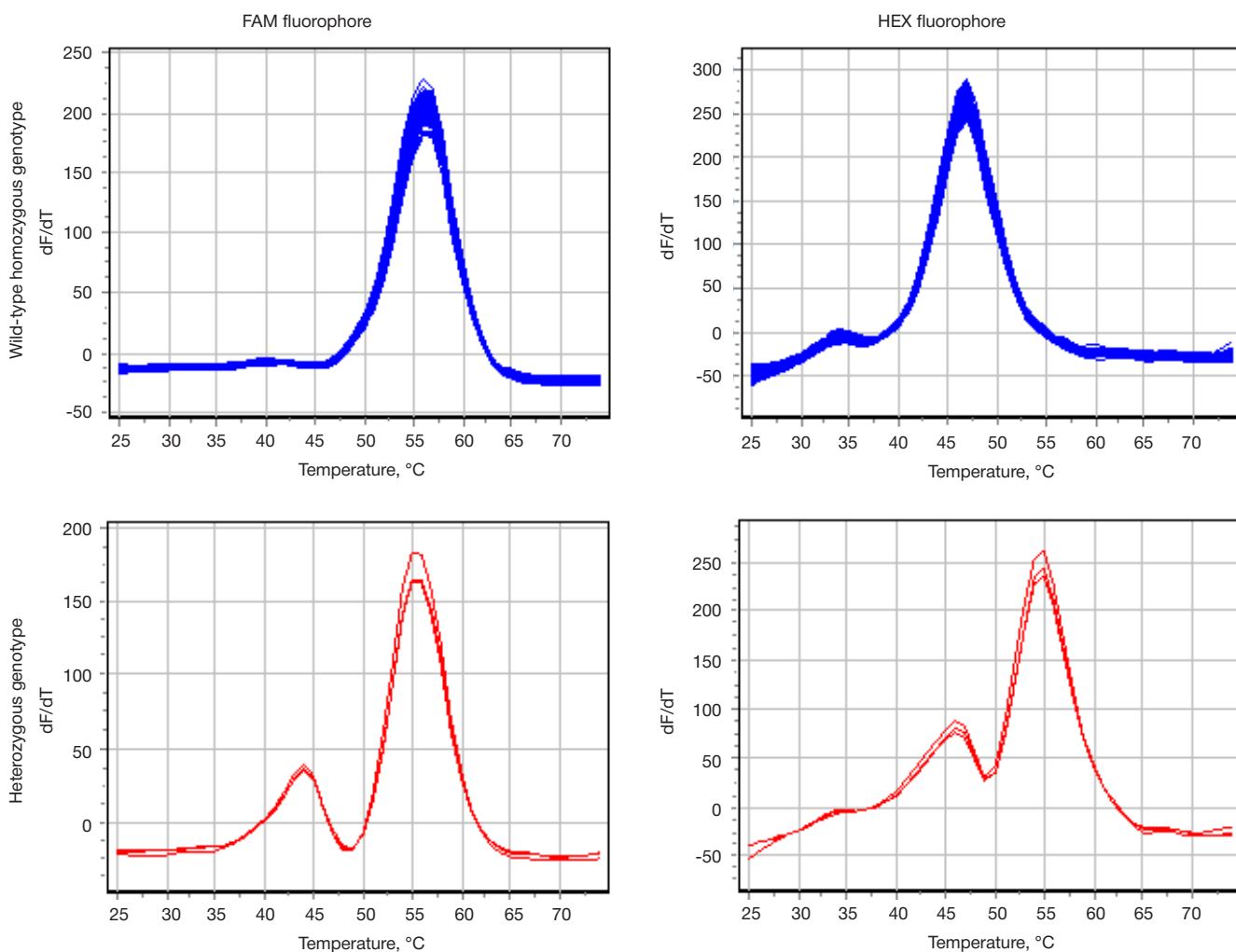
DNA was extracted from 0.1 ml peripheral blood using the reagent kit "PROBA-GS-Genetics" (DNA-Technology, Russia). The extraction method we used involves lysis of the biomaterial followed by DNA binding to silica support, washing, and DNA elution. Some of the obtained DNA samples were immediately

used for genotyping; others were stored under  $-20\text{ }^{\circ}\text{C}$ . The average DNA concentration measured by the Qubit flourometer (Invitrogen, USA) was 50–100  $\mu\text{g/ml}$ .

To detect single nucleotide substitutions, we used the reagent kit "Screening for monogenic diseases" (DNA-Technology, Russia). The detection principle relies on the method of adjacent (or kissing) probes [8, 9]. The kit can identify 5 *GJB2* mutations associated with nonsyndromic sensorineural hearing loss and 1 *GALT* mutation associated with galactosemia.

Each reagent kit includes amplification mixes for identifying an individual mutation. Each mix contains primers complementary to both wild-type and mutant nucleotide sequences, one quencher-labeled oligonucleotide and two sequence-specific fluorophore-labeled oligonucleotide probes. The oligonucleotide probes complementary to wild-type and mutant sequences are labeled with different fluorophores, which makes it possible to simultaneously identify both variants using one test tube.

The first step in the identification of single nucleotide substitutions was PCR. Then the temperature of the reaction mix was brought down to hybridize oligonucleotide probes to the obtained matrices. Genotyping was performed following PCR and hybridization by changing fluorescence intensity during heat-induced denaturation of the oligonucleotide duplexes and the obtained matrices. The measurements were carried out in real time; based on the results, melting curves



Melting curves for two genotype variants based on the data obtained during the detection of Q188R mutation in the *GALT* gene

were constructed (see the image below). If an analyzed sample contained only one variant of a nucleotide sequence, i. e., the studied polymorphism was homozygous, the melting point for a perfect match probe/target duplex was considerably higher than for a mismatched duplex. If a heterozygous sample was analyzed that contained both variants of the nucleotide sequence, then both probes formed a perfect match duplex, which is why their melting points were practically the same.

This approach certainly has advantages over most molecular-genetic techniques for the detection of single nucleotide polymorphisms, including TaqMan assays. Here, genotyping is performed twice and data are obtained independently from two fluorescence channels, which significantly increases the reliability of the genotyping procedure and is highly unlikely to be reproduced using other techniques.

Polymerase chain reaction and measurements of oligonucleotide probe melting points were performed using the detecting amplifier DTprime (DNA-Technology, Russia). The following temperature mode was used for amplification: 94 °C for 10 s, 64 °C for 30 s; number of cycles = 50. After amplification was completed, the reaction mix was cooled down to 25 °C at a rate of 2 °C/s. Melting curves were constructed from the data obtained with the following technique: the temperature of the reaction mix was increased incrementally from 25 to 75 °C (1 °C per heating step); fluorescence was measured at each heating step. We used domestic equipment to automate the main stages of the research, and thus were able to genotype 40 mutations in up to 100 samples a day.

For control, we performed selective automated Sanger sequencing using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA) and reagents by the same manufacturer. Control tests demonstrated the results identical to those obtained in our experiment.

## RESULTS

Frequencies of *GJB2* and *GALT* mutations detected in 1000 healthy first-time blood donors who identified themselves as Russians and permanently reside in the Russian Federation are shown in the table below. Genotyping of these individuals identified 37 carriers of *GJB2* mutations associated with sensorineural hearing loss (frequency in the sample was 3.7 %, or 1 : 27) and 6 carriers of *GALT* mutations associated with galactosemia (frequency in the sample was 0.6 %, or 1 : 167). One individual carried both mutations. In total,

42 carriers of *GJB2* and *GALT* mutations were detected (frequency in the sample was 4.2 %, or 1 : 24).

## DISCUSSION

Our research identified a total of 42 carriers of *GJB2* and *GALT* mutations (frequency in the sample was 4.2 %, or 1 : 24). Besides, one individual was found to carry both mutations. The results of the present study are in agreement with the published research carried out in the Russian population [1, 3–5]. However, we detected a slightly higher (compared to the average data on the European population) frequency of the *GJB2*:35DELg mutation associated with sensorineural hearing loss.

## CONCLUSIONS

In this research study, we determined frequencies of *GJB2* and *GALT* mutations in healthy Russian individuals. In total, 42 carriers of these mutations were detected (frequency in the sample was 4.2 %, or 1 : 24). These data indicate a relatively high prevalence of inherited conditions and provide a rationale for introducing molecular-genetic diagnostic tests into clinical practice (in addition to neonatal screening) that can assist pregnancy planning and be a criterion for the use of reproductive technologies in infertile patients.

The most suitable platform for such research is real time PCR. This approach may open up new horizons for mass high throughput sequencing, facilitate automation of laboratory work and help to achieve highly accurate and reliable results.

Heterozygous variants of *GJB2* and *GALT* mutations and concurrent mutations in 1000 Russian first-time blood donors

Mutation	Number of heterozygous variants detected
<b>In <i>GJB2</i></b>	
35delG	37
167delT	0
235delC	0
313_326del14	0
358_360delGAG	0
<b>In <i>GALT</i></b>	
Q188R	6
<b>Concurrent mutations</b>	
<i>GJB2</i> :35delG + <i>GALT</i> :Q188R	1

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