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SHORT COMMUNICATIONS

Reconstruction of SNP Haplotypes with Mutation c.-23+1G>A in Human Gene *GJB2* (Chromosome 13) in Some Populations of Eurasia

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Abstract—The c.-23+1G>A splice site mutation is one of the most frequent mutations of gene *GJB2* (Cx26, 13q11-q12) associated with congenital non-syndromic autosomal recessive deafness. This mutation is characterized by a wide spread from Eastern Siberia and Central Asia to Eastern Europe, the Middle East, and South Asia. It is currently unknown whether this mutation spread over such a vast territory as a result of the founder effect or there were several local centers of origin of this mutation. For the first time, on the basis of the analysis of variability of nine SNP markers, five different haplotypes in deaf patients homozygous for mutation c.-23+1G>A from six Eurasian populations were reconstructed. The structure of the haplotypes revealed in Yakuts, Russians, Evenks, Tuvinians, Mongols, and Turks makes it possible to assume that mutation c.-23+1G>A (*GJB2*) could have spread across Eurasia as a result of the founder effect. The greatest diversity of haplotypes with c.-23+1G>A was found in patients from Mongolia, which probably refers to the earlier period of expansion of haplotypes carrying this mutation on the territory of Central Asia.

Keywords: gene *GJB2*, mutation c.-23+1G>A, founder effect, Eurasia
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Mutations of gene *GJB2* (MIM 121011, 13q11-q12), which encodes the protein connexin 26 (Cx26), are the main cause of congenital non-syndromic hearing loss and deafness in many countries of the world. Currently, more than 300 pathogenetic variants in this gene are known [1]. It is known that the prevalence of individual major *GJB2* mutations has ethnogeographic specificity, in many cases due to the founder effect, and for some major mutations, founder haplotypes were found and estimates of their age were obtained: c.35delG (Europe, mutation age ~10000 years), c.167delT (Ashkenazi Jews, mutation age is not defined), c.235delC (East Asia, mutation age ~11500 years), p.Trp24* (India, mutation age ~7880 years), and p.Val37Ile (Southeast Asia, mutation age ~7500 years) [2–6]. The world highest frequency of mutation c.-23+1G>A, which affects the conservative base of the donor splice site of gene *GJB2*, was detected in Yakutia [7]. In addition, for the Yakut population, genotyping of seven STR markers revealed a common founder haplotype carrying the mutation c.-23+1G>A, whose age of expansion is estimated at about 800 years [7]. Using another panel of genetic markers (nine SNPs), commonality of haplotypes with mutation c.-23+1G>A has also been shown for deaf patients homozygous for this mutation from Mongolia and Turkey [8]. In addition to Yakuts, Mongols, and Turks [7, 8], mutation c.-23+1G>A is

Table 1. Characteristics of samples

	Number of homozygotes for mutation c.-23+1G>A	Anthropological affiliation (race/type*)	Linguistic affiliation (family/group)	Region	Reference
Yakuts	111	Asian/ Central Asian	Altaic/Turkic	Republic of Sakha (Yakutia), Eastern Siberia, Russia	This work
Russians	1	Caucasian/ Eastern European	Indo-European/ Slavic	Vladimir oblast, Eastern Europe, Russia	″
Evenks	1	Asian/ Baikal	Altaic/Tungusic	Republic of Sakha (Yakutia), Eastern Siberia, Russia	″
Tuvinians	6	Asian/ Central Asian	Altaic/Turkic	Republic of Tuva, Southern Siberia, Russia	″
Mongols	4	Asian/ Central Asian	Altaic/Mongolian	Mongolia, Central Asia	[8]
Turks	3	Caucasian/ Mediterranean	Altaic/Turkic	Turkey, Middle East	″

* Dominant anthropological type.

now found in deaf individuals from various regions of the world: in Europe [9–15], the Caucasus [16], the Middle East [17–20], and India and Bangladesh [5, 21]; and recently it was found in South Siberia (Tuvinian population) [22]. However, up to the present there have been no studies on the reconstruction of haplotypes bearing the mutation c.-23+1G>A carried out using an unified system of genetic markers that would answer the following question: Did this mutation spread across Eurasia as a result of the founder effect or could it have had several local centers of origin?

The aim of this work is the reconstruction of haplotypes with mutation c.-23+1G>A of gene *GJB2* on the basis of analysis of nine SNP markers in deaf patients homozygous for this mutation.

For analysis of haplotypes with mutation c.-23+1G>A, the following SNP markers flanking the *GJB2* gene at a distance of ~13.4 kb were used: rs1932429, rs5030702, rs7987144, rs7994748, rs4769974, rs2274084, rs2274083, rs11841024, and rs2313477 [8]. The choice of these SNP markers was determined by the possibility of a comparative analysis with previously published data on Mongolia and Turkey [8]. The studied sample of deaf patients is 119 individuals with mutation c.-23+1G>A in the homozygous state: Yakuts (n = 111), Tuvinians (n = 6), Evenk (n = 1), and Russian (n = 1) (Table 1).

For the study, genomic DNA samples extracted from peripheral blood lymphocytes were used. Amplification of DNA was carried out by PCR on a programmable Thermal Cycler (Bio-Rad). Genotyping of seven SNP markers was carried out by PCR-RFLP analysis, with enzymatic processing of PCR products with restriction endonucleases from SibEnzyme (rs1932429, *Taq*I; rs5030702, *Acc*36I; rs7987144, *Alu*I; rs7994748, *Msp*20I; rs4769974, *Kzo*9I; rs11841024, *Alu*I; rs2313477, *AccB*7I). Genotyping of SNP markers rs2274084 and rs2274083 was carried out by sequencing on the Genetic Analyzer ABI Prism 3130XL (Applied Biosystems). Sequences were analyzed using the program Chromas v. 2.0. The frequencies of probable haplotypes in the study group of individuals were calculated using the EM algorithm (Arlequin v. 3.1). Phylogenetic analysis of SNP haplotypes was carried out using the maximum likelihood method (Network 5.0).

The results of genotyping of SNP markers and the frequency of occurrence of haplotypes in the sample compared with the published data are presented in Table 2. Haplotype analysis of chromosomes with mutation c.-23+1G>A of gene *GJB2* revealed five different haplotypes: no. 1 (ATACCAGAC), no. 2 (GTACCAGAC), no. 3 (GTACCGGAC), no. 4 (GTACCGGCC), and no. 5 (GTATCGGAC) (Table 2). The greatest variety of haplotypes was found in patients from Mongolia (four different haplotypes) and Yakutia (three different haplotypes). All other deaf patients (Tuvinians, Russian, Evenk, and Turks) are represented by only haplotype no. 2.

The phylogenetic analysis was carried out using the data on the frequencies of haplotypes in the studied sample of deaf patients (chromosomes with mutation, n = 252) and frequencies of haplotypes of 5070 chromosomes without mutation from different populations of the world (initial data were taken from the 1000 Genomes Project) reconstructed according to data on variability of the nine SNP markers studied.

The phylogenetic analysis showed that the ancestral haplotype is probably the relatively rare haplotype no. 3, which was found on chromosomes only in Yakuts and Mongols, but not haplotype no. 2, major for all studied populations (Fig. 1). The results indicate that the basic haplotype, from which the mutant

Table 2. Genotypes for nine SNP markers, reconstructed haplotypes, and their frequencies in the studied populations

Population	Number of individuals	rs2313477	rs11841024	rs2274083	rs2274084	rs4769974	rs7994748	rs7987144	rs5030702	rs1932429
Yakuts	109	*GG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
	1	*AG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
	1	*GG*	*TT*	*AA*	*CC*	*CC*	*GA*	*GG*	*AA*	*CC*
Tuvinians	6	*GG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
Evenk	1	*GG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
Russian	1	*GG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
Mongols [8]	1	*GG*	*TT*	*AA*	*CC*	*CC*	*GA*	*GG*	*AA*	*CC*
	1	*GG*	*TT*	*AA*	*TC*	*CC*	*GA*	*GG*	*AA*	*CC*
	1	*GG*	*TT*	*AA*	*CC*	*CC*	*GA*	*GG*	*CA*	*CC*
	1	*GG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
Turks [8]	3	*GG*	*TT*	*AA*	*CC*	*CC*	*AA*	*GG*	*AA*	*CC*
Major allele		*G*	*T*	*A*	*C*	*C*	*A*	*G*	*A*	*C*

Haplotype frequency, %

Haplotype	Haplotype no.	Mongols [8] ($n = 8$)	Yakuts ($n = 222$)	Tuvinians ($n = 12$)	Evenks ($n = 2$)	Russians ($n = 2$)	Turks [8] ($n = 6$)	Total chromosomes ($n = 252$)
A T A C C A G A C	1	–	0.45	–	–	–	–	0.04
G T A C C A G A C	**2**	**62.50**	**99.10**	**100.00**	**100.00**	**100.00**	**100.00**	**98.00**
G T A C C G G A C	3	12.50	0.45	–	–	–	–	0.08
G T A C C G G C C	4	12.50	–	–	–	–	–	0.04
G T A T C G G A C	5	12.50	–	–	–	–	–	0.04

Homozygous genotypes are indicated by gray; the most frequent haplotype is in bold; n is the number of chromosomes studied.

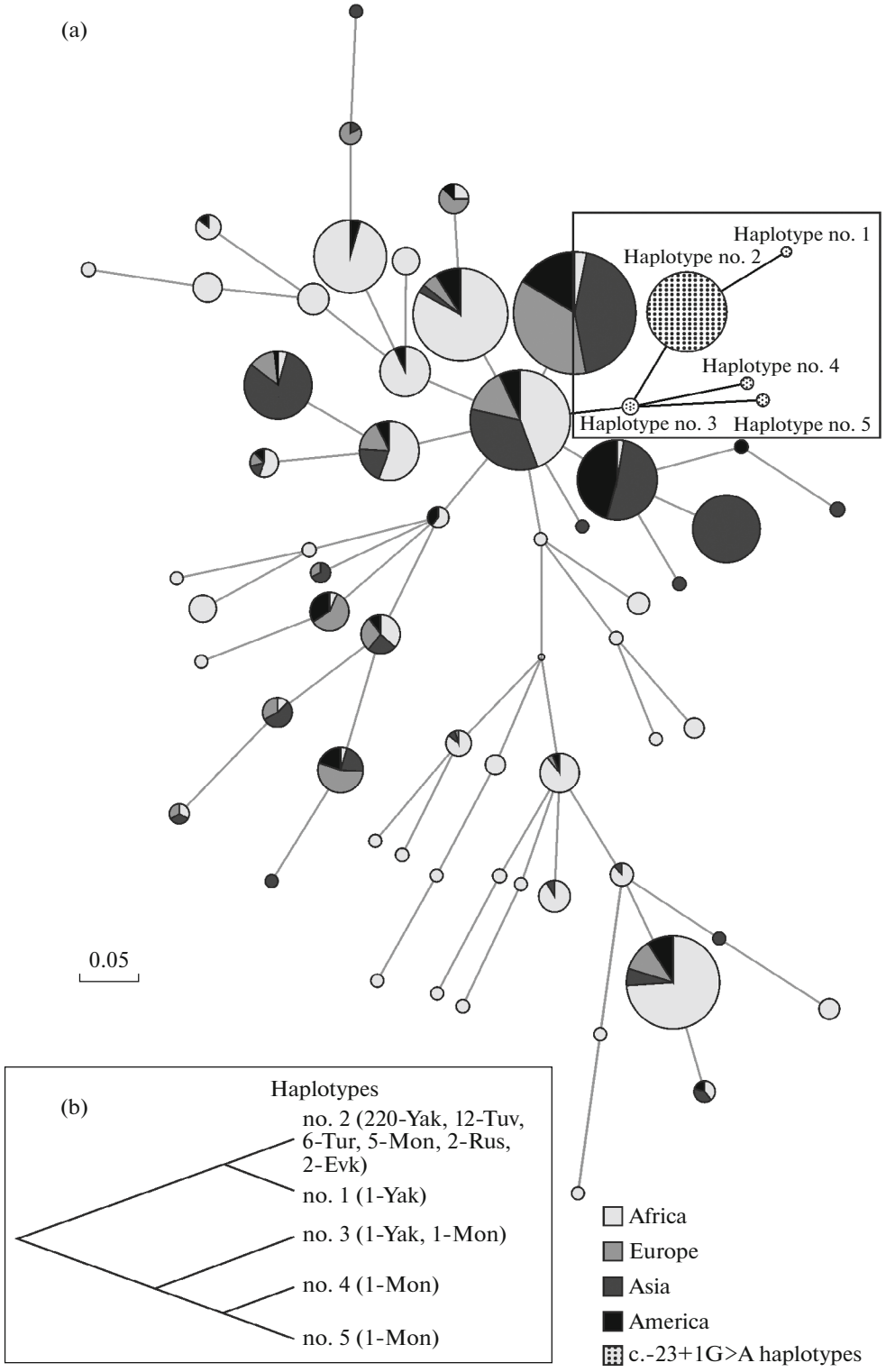

Fig. 1. Phylogenetic analysis of haplotypes with mutation c.-23+1G>A of gene *GJB2* (Network 5.0). (a) Haplotypes are indicated by circles; the area of a circle is proportional to the haplotype frequency. Haplotypes without mutation were reconstructed according to the data of the 1000 Genomes Project. **Africa**—ACB, ASW, ESN, GWD, LWK, MSL, and YRI; **Europe**—CEU, FIN, GBR, IBS, PJL, and TSI; **Asia**—BEB, CDX, CHB, CHS, GIH, ITU, JPT, KHV, and STU; **America**—CLM, MXL, PEL, and PUR. The three-letter abbreviations referring to the studied populations are explained in the open database of 1000 Genomes (http://www.1000genomes.org) [23]. (b) The dendrogram of haplotypes with mutation c.-23+1G>A. The dendrogram is constructed using the maximum likelihood method. Analysis includes haplotypes of 252 chromosomes with mutation c.-23+1G>A of gene *GJB2* and haplotypes of 5070 chromosomes from various populations of the world (1000 Genomes). Yak, Yakuts; Tuv, Tuvinians; Tur, Turks; Mon, Mongols; Rus, Russian; Evk, Evenks.

cluster of haplotypes with mutation c.-23+1G>A has separated, is not specific to certain populations of the world (typical of African, Asian, European, and American populations) (Fig. 1a), which does not make it possible to unequivocally determine the initial center of origin of this mutation. However, the greatest haplotype diversity was observed in deaf patients from Mongolia, and it can be assumed that one of the most ancient regions of the spread of mutation c.-23+1G>A was the territory of Central Asia. In general, the similar structure of haplotypes with mutation c.-23+1G>A of gene *GJB2* in Yakuts, Russians, Evenks, Tuvinians, Mongols, and Turks suggests that this mutation could have spread across Eurasia as a result of the founder effect. However, the studied SNP markers do not have high variability, so the issue of migration routes of the c.-23+1G>A mutation carriers requires further in-depth study with an increase in study resolution and sizes of the sample of patients with mutation c.-23+1G>A of gene *GJB2* in a homozygous state from different populations of the world.

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