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Frequencies of Y Chromosome Binary Haplogroups in Belarussians

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Abstract—The compositions and frequencies of Y-chromosome haplogroups identified by genotyping 23 biallelic loci of its nonrecombining region (*YAP, 92R7, DYF155S2, 12f2, Tat, M9, M17, M25, M89, M124, M130, M170, M172, M174, M173, M178, M201, M207, M242, M269, P21, P25*, and *P37*) have been determined in a sample of 68 Belarussians. Eleven haplogroups have been found in the Belarussian gene pool (E, F*, G, I, I1b, J2, N3a*, Q*, R1*, R1a1, and R1b3). Haplogroup R1a1 is the most frequent; it includes 46% of all Y chromosomes in this sample. The frequencies of haplogroups I1b and I are 17.6 and 7.3%, respectively. Haplogroup N3a* is the next in frequency. The frequencies of haplogroups E, J2, and R1b3 are 4.4% each; that of R1* is 3%; and those of F*, G, and Q* are 1.5% each.

INTRODUCTION

Analysis of Y-chromosome haplogroups obtained by genotyping a set of DNA markers of its nonrecombining region is one of the main approaches to studying the structure of the gene pools of modern populations and their genetic history [1]. Studies using mtDNA [2, 3] and Y-chromosome [4, 5] markers have demonstrated that the genetic legacy of the Paleolithic European human population accounts for 80% of the modern European gene pool, and only 20% of it is the contribution of Neolithic migrants from Near East. The geographic gradients of haplogroup frequencies in population gene pools reflect past migrations differing in time and direction. To date, detailed analysis of the genetic structures of various ethnic groups in order to determine in detail their specific features seems relevant. In works published during several past years, Y-chromosome markers were used to study the populations of Scandinavia [6], Anatolia [7], the British Isles [8], the Carpathian region [9], three largest Mediterranean islands [10], the Caucasus [11], and other European regions. Some other studies focused on individual ethnic groups, including Icelanders [12], Irish [13], Armenians [14], Saami [15], and various Finno-Ugric populations [16]. However, the male gene pool structure of Eastern European populations, including Eastern Slavs, has been studied poorly.

We studied the distribution of haplogroups identified by genotyping 23 biallelic loci of the Y-chromosome nonrecombining region (*YAP, 92R7, DYF155S2, 12f2, Tat, M9, M17, M25, M89, M124, M130, M170, M172, M174, M173, M178, M201, M207, M242, M269, P21, P25*, and *P37*) in a sample of Belarussians. The sample consisted of persons that were unrelated to one another in the paternal line in at least three past generations.

MATERIALS AND METHODS

The material consisted of total DNA isolated from peripheral blood lymphocytes by a standard method. A total of 68 blood samples were analyzed.

Loci *YAP, 92R7, DYF155S2, 12f2, Tat, M9, M17, M25*, and *M89* were genotyped as described earlier [17].

Markers M170 and M174 were genotyped via directly sequencing polymerase chain reaction (PCR) products obtained by means of an ABI Prism 310 automated analyzer (Perkin Elmer) with the use of primers described earlier [18] and a BigDye Terminator cycle sequencing kit as recommended by the manufacturer.

The other loci were genotyped using PCR in a Tertsik thermocycler (DNK-Tekhnologiya) followed by analysis of the restriction-fragment lengths (for *M130, M172, M173, M178, M207, M242, M269*, and *P37*) or using allele-specific PCR (for *M124, M201, P21*, and *P25*).

Restriction endonucleases and ready-made buffer solutions were from SibEnzim (Novosibirsk); the reactions were carried out as recommended by the manufacturer. All primer sequences were taken from the nomenclature system [18] unless indicated otherwise. Electrophoresis was performed in 3% agarose gel (for *M172, M178*, and *M207*), 10% PAAG (for *M173*), or 2% agarose (for other markers).

Marker *M130,* which is a C–T transition, was genotyped by the restriction analysis of PCR products. The T allele was detected by the presence of a *Bsc4*I restriction site at which the 205-bp PCR product is cleaved into a 159-bp and a 46-bp fragments.

For typing the T–G transversion (mutation *M172*), we used a modified reverse primer (R: 5'-TAA TAA TTG AAG ACC TTT TGA GT-3'), which, in the case of the G allele, resulted in the appearance of a *Hinf*I site and the cleavage of the 220-bp PCR product into a 197-bp and a 23-bp fragments.

Marker *M178* (a C–T transition) was genotyped similarly, with the use of a modified reverse primer (M178R: 5'-AGT TCT CCT GGC ACA CTA AGG AGC C-3'), which, in the case of the T allele, resulted in the appearance of a *Bsp19*I site and the cleavage of the 245-bp fragment into a 218-bp and a 27-bp ones.

Marker M207 (an A–G transition) was detected by the presence of a *Dra*I restriction site in the A allele (a 346-bp and a 77-bp fragments) and its loss in the case of mutation (a 423-bp fragment).

Marker *M242* (a C–T transition [19]) was detected by the presence of a *Bbv12*I restriction site in the C allele (a 179-bp and a 187-bp fragments) and its loss in the case of mutation (a 366-bp fragment).

The allelic state of marker *P37* (a T–C transition) was determined by the absence of a *Bst4C*I restriction site in the T allele (447 bp) and its appearance in the case of mutations (136 bp and a 311 bp).

For typing the *M269* marker, which is a T–C transition [20], we used the *Bst2*UI restriction endonuclease. Its restriction site was absent in the T allele (427 bp) and its presence in the C allele (357 bp and 68 bp).

For typing the A–C transversion (mutation *M173*), we used a modified reverse primer (R: 5'-TCT GAA TAT TAA CAG ATG ACA CAG-3'), which, in the case of the G allele, resulted in an additional *Bst4*CI restriction site and the cleavage of the 215-bp fragment into four parts (91, 69, 33, and 22 bp). In the case of the A allele, restriction yielded only three fragments (91, 91, and 33 bp).

Allele-specific PCR was used for genotyping the *M124, M201, P21*, and *P25* markers. The *M124* mutation is a C–T substitution. Each of the two reverse primers was strictly complementary to only one of the allelic variants and differed from the other primer in one nucleotide at the 3'-end (M124R1: 5'-CAC AAA CTC AGT ATT ATT AAA CCA-3' and M124R2: 5'-CAC AAA CTC AGT ATT ATT AAA CCG-3'). The 269-bp fragment was present only in one PCR variant, at an annealing temperature of 63°C.

Marker *P21* a C–A substitution) was genotyped by a similar method. We used two different reverse primers (P21R1: 5'-GTG AGG TGT CAG AGC AGG AGA GTA G-3' and P21R2: 5'-GTG AGG TGT CAG AGC AGG AGA GTA T-3'). The 780-bp fragment was present only in one PCR variant, at an annealing temperature of 65°C.

For typing the G–T transversion (marker *M201*), we used two different forward primers (M201F1: 5'-CTA ATA ATC CAG TAT CAA CTG AGG G-3' and M201F2: 5'-CTA ATA ATC CAG TAT CAA CTG AGG T-3'). The annealing was performed at 66°C. The 215-bp fragment was found in only one PCR variant.

Marker *P25* was genotyped by a similar method using two different forward primers (P25F1: 5'-TAT CTG CTG CCT GAA ACC TGC CTG C-3' and P25F2: 5'-TAT CTG CTG CCT GAA ACC TGC CTG A-3'). The 269-bp fragment was present only in one PCR variant, at an annealing temperature of 58°C.

In some blood samples, all biallelic markers were genotyped; usually, however, we performed genotyping hierarchically on the basis of the known sequence, in which mutations had accumulated in the Y chromosome [1, 18]. The haplogroups were designated according to the standard nomenclature suggested by the Y-Chromosome Consortium [18].

For visualization of the amplified fragments and video recording of the gels, we used the gel documentation and analysis system from the Advanced American Biotechnology and the following software: Video Studio v.1.0 (Ulead Systems Inc.), Video Packer Plus v.1.2p (Aura Vision Corp. & VIC Hi Tech Corp.), and Adobe Photoshop v.6.0 (Adobe Systems Inc.).

RESULTS AND DISCUSSION

The analysis of allele distribution for 23 loci of the nonrecombining region of the Y chromosome in 68 Belarussians revealed 11 haplogroups (table). The spectrum of markers was greater than in our previous study [17], where a sample of Ukrainians was analyzed. This allowed us to identify novel haplogroups and revise the data on some of previously known ones.

Haplogroup R1a1 was the most frequent Y-chromosome variant in the sample studied. In the Ukrainian [17] and Russian [21] sample studied earlier, the frequencies of this haplogroup were practically equal (43.6 and 40.6%, respectively). Almost the same frequencies were earlier found in other Slavic and in Baltic populations [4, 8], which apparently reflects their genetic relationship. Probably, these Eastern European ethnic groups are especially close to ancient Indo-Europeans associated with the pit and mound archeological cultures, which are associated with the spread of R1a1 [8, 21].

Haplogroup I1b was the second most frequent in the Belarussian sample. The frequency of this haplogroup is the highest in Bosnians and Croatians (30–40%); these populations are also characterized by the most diverse microsatellite haplotypes within this haplogroup [22]. It is presumed that the carriers of haplogroup I1b spread and formed its modern geographic area when Europe was peopled again from the Balkan

Frequencies of Y-chromosome haplogroups found in the Belarussians, %

	E	F*	G	I	I1b	J2	N3a*	Q*	R1*	R1a1	R1b3	Σ*N*
Data of this study	4.4 (3)	1.5 (1)	1.5 (1)	7.3 (5)	17.6 (12)	4.4 (3)	8.8 (6)	1.5 (1)	2.9 (2)	45.6 (31)	4.4 (3)	68
Data from the study [4]	10 (4)	34 (14)				2 (1)	2 (1)			39 (16)		41

Note: The number of individuals is indicated in parentheses, Σ*N* is the total sample size. In study [4] Y-chromosomes of five Belarussians can't be classified within the limits of modern system of terms of Y-chromosome haplogroups.

refugium, mainly eastwards, after the last peak of glaciation.

Haplogroup N3a* ranked third in frequency in the sample studied. The spread of this haplogroup in Eastern Europe was undoubtedly related to the westward expansion of Finno-Ugric tribes from the Cis-Ural region [16]. Other European ethnic groups, whose gene pools initially had no N3a*, acquired it as they assimilated Finnish tribes while spreading eastwards. This mainly applies to Slavic, Baltic, and Scandinavian populations.

Thus, these three haplogroups were the main components of the Y-chromosome gene pool of the sample studied and together account for 72% of the total number of Y chromosomes. Similar frequencies of these haplogroups were earlier found in the Ukrainian population [17] (I1b was not differentiated from F in that study).

The frequency of haplogroup I (I*xP37*) was slightly higher than 7%. Most probably, these blood samples belong to the I1a haplogroup that we did not differentiate; it is presumed to mark (similarly to I1b) the postglacial expansion of humans from the refugium located on the Iberian Peninsula. Its proportion is the highest in Germanic ethnic groups of Scandinavia (25–35%), in which the I1b haplogroup has not been found [22]. In the Eastern Slavic gene pool, both groups of migrants seem to have left a trace.

Haplogroups E, J2, and R1b3 were found in three blood samples each, so the frequency of each of them was lower than 5%. Haplogroups F*, G, Q*, and R1* were found in one blood sample each (frequencies of several percent). Haplogroups E, J2, and G mark the spread of Neolithic farmers from Near East [4, 7, 8, 21, 23]. Haplogroup R1b3, which is very common in European populations, was spread over Europe in Upper Paleolithic Age, presumably by the people associated with the Aurignacian culture [7, 8]. Haplogroups Q* and R1* have also come from Upper Paleolithic migrants [7, 19].

The composition and frequencies of Y-chromosome haplogroups in the Belarussian gene pool are similar to those in the gene pools of other Eastern Slavic populations (Ukrainians and Russians) studied earlier [17, 21], except for haplogroup J2, the frequency of which in Ukrainians was two times higher (11.7%). We continue to analyze the structure of the gene pools of Slavic populations with the use of Y-linked microsatellite markers. This will supplement the existing data on the diversity of STR markers in these populations [24] and permit a more detailed analysis of the structure of the biallelic haplogroups that we found in Russians, Ukrainians, and Belarussians.

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