Y-haplogroup frequencies in the Slovak Romany population

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Abstract European ‘gypsies’, commonly referred to as Romanies, are represented by a large number of groups spread across many countries. We performed a population genetic study on 200 unrelated Romany males to reveal the genetic origin of the Slovak Romany population. On the basis of Y-chromosome haplotypes, we determined the corresponding Y-haplogroups using Whit Athey’s Haplogroup Predictor. The obtained distribution of haplogroups provided strong evidence of Asian origins, especially Indian. The Indian Y-haplogroup H was the most prevalent and represented 40% of all the samples. The distribution of haplogroups was: E1b1b, 21%; J2, 16.5%; I1a, 14%. Haplogroups R1a, R1b, I2a, and N1 were observed in small frequencies. The obtained genetic structure indicated that the endogamous Romany population has been shaped by a genetic drift and differential admixture, and correlates with the migratory history of the Romanies in Europe.

Key words: Y-chromosome, Y-haplogroups, Romany ‘gypsy’ population, eastern Slovakia, Indian origin

Introduction

The non-recombining portion of the human Y-chromosome provides anthropologists and geneticists with an extremely powerful tool for historical and demographic studies (Cavalli-Sforza et al., 1994). The number of studies about Short Tandem Repeats on the Y chromosome (Y-STR) increases greatly every year, but to evaluate their efficiency in forensic and anthropological sciences, it is necessary to investigate a large number of different populations (Turrina et al., 2006). Y-STR markers can be used for paternity testing, male kinship analysis, for evolutionary studies, and for studies of human migration (Butler, 2003).

There is also considerable interest in determining the Y-chromosome haplogroup, a group or a family of Y-chromosomes related by descent. Determination of the Y-haplogroups by direct testing of single nucleotide polymorphisms (SNPs) can sometimes be an expensive process. Therefore, there is much interest in predicting the haplogroups from a set of Y-STR markers (Athey, 2005). We estimated frequency of the major Y-chromosome haplogroups from acquired Y-STR data by use of the web-accessible program, Whit Athey’s Haplogroup Predictor, which is based on a Bayesian-allele-frequency approach. The program computed the probability that a Y-STR haplotype was in a haplogroup (Athey, 2006). Identification of Romany Y-chromosome haplogroups yields the ancestry of the paternal line of the population being studies.

Population

Approximately 350,000–380,000 Romanies live in Slovakia according to the official statistical list. The highest concentration (54%) of the Romany population is located in eastern Slovakia (Vaňo, 2001). The Romany population of Slovakia represents a genetically isolated population with a high frequency of consanguinity and inbreeding, which is about 10–100 times higher than in the European population of the same region (Ferák et al., 1987). Several studies of various genetic markers in different Romany samples from Slovakia showed that their gene pool differs from the Slovak majority population and neighboring European populations (Nagy et al., 2006; Soták et al., 2008; Malyarchuk et al., 2008). The population differentiation analysis based on autosomal STR markers between Slovak Romanies and Europeans showed significant differences in spite of their geographical proximity (Soták et al., 2008). The Romany population is characterized by nomadism, a caste structure and its position in numerous countries as an underprivileged ethnic minority (Morar et al., 2004).

The first reference to Romany people in Slovakia is from the 14th century in the region of Spiš. The origin of the Romany population living in Slovakia has not yet been satisfactorily explained (Bernasovský and Bernasovská, 1999). Cultural anthropology, linguistics, and limited historical records from the surrounding majority population describe the ‘gypsies’ as a population of Indian origin, with their exodus from India dating approximately from the 5th–10th centuries AD, their arrival in Byzantium dating from the 11th or 12th centuries, and their dispersal throughout Europe documented at the end of the 15th century (Fraser, 1992; Marushiakova and Popov, 1997).

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Methods

Samples

Genomic DNA of 200 healthy and unrelated Slovak Romany males was extracted from buccal swabs using the Jet Quick DNA tissue kit according to the manufacturer's instructions (Genomed). Written informed consent was obtained from all participants.

Y-typing and data analysis

The multiplex polymerase chain reaction (PCR) reaction was performed for each DNA sample by use of the PowerPlex® Y System according to the supplier's protocol (Promega). This kit coamplifies 12 STRs of the Y chromosome (Y-STRs), including the markers defined as the 'European minimal haplotype' (Gill et al., 2001) (DYS19, DYS385 a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393), plus two loci (DYS438 and DYS439) added to this panel by the Scientific Working Group on DNA Analysis Methods (SWGDAM) (Lee et al., 2004) and DYS437. PCR products were detected by a MegaBACE 1000 genetic analyzer. MegaBACE Genetic Profile software was used to collect data and to analyze fragment sizes. Y-STR alleles were named according to the number of repeat units they contain according to the recommendations of the DNA Commission of the International Society for Forensic Genetics (Bär et al., 1997). The discrimination capacity was calculated as the number of the different haplotypes divided by the number of individuals. Prediction of Y-chromosome haplogroups from Y-STR values was performed using Whit Athey's Haplogroup Predictor Program, version 5, which is based on a Bayesian-allele-frequency approach (Athey, 2006). Twelve Y-STR markers were input and the Bayesian probability for each haplogroup was estimated. The Y-haplogroup nomenclature used in this paper was according to the recommendations of the Y-chromosome consortium (YCC) (2008).

Results and Discussion

The genetic analysis of 200 Romany samples showed 83 different haplotypes; 61 (30.5%) occurred in only one copy. The most frequent haplotype 10-12-12-28-10-16-14-11-13-22-14/14 was found in 22 copies. The gene diversity in the analyzed population was 0.9623 ± 0.0057 and the discrimination capacity was 41.5%.

We predicted Y-chromosome haplogroups from Y-STR data by the use of Haplogroup Predictor program. Table 1 and Figure 1 show the distribution of Y-haplogroups in the Slovak Romany population. The observed haplotypes, predicted haplogroups of the Romany population and the Bayesian probability are reported in Table 2. We found only eight haplogroups from the core 15 haplogroups in the studied population. Four haplogroups—namely H, E1b1b, J2, I1a—occurred in high frequencies (>10%) and together accounted for ~90% of all Y-chromosomes. Haplogroups R1a, R1b, I2a, N1 were observed in small numbers (representing 0.5–4.5%). The remaining haplogroups, E1b1a, I1b2a, J1, G2a, G2c, L, and Q, were not detected in the samples studied. The Bayesian probability was greater than 60% in all of the samples.

Table 1. Distribution of Y-haplogroups in Slovak Romany population ($n = 200$)

Haplogroup	Number	Percent
E1b1b	42	21
H	80	40.0
I1a	28	14
I2a	1	0.5
J2	33	16.5
N1	1	0.5
R1a	6	3
R1b	9	4.5
Total	200	100

Figure 1. Graphical presentation of Y-haplogroup frequencies from Table 1.

Haplogroup H was found to be the most prevalent Y-lineage and represented 40% (80 individuals) of all the samples. According to the International Society of Genetic Genealogy, haplogroup H is typical of the Indian subcontinent area. Y-haplogroups were analyzed in three tribal populations from Andhra Pradesh in southern India and haplogroup H was the most prevalent with frequencies of 0.30 (Thanseem et al., 2006). Studies of Bulgarian, Macedonian, and Vlach Romanies showed that 44.8%, 59.6%, and 73%, respectively, obtained haplotypes belonging to Y-haplogroup H (Gresham et al., 2001; Kalaydjieva et al., 2001b; Pericic et al., 2005). According to these results, the Romany population in Slovakia shares a common ancestry with Romany populations from neighboring countries. Haplogroup H has been found very rarely in non-Romany populations and populations outside the Indian subcontinent (Wells et al., 2001). The Romany populations in Europe are the main source of haplogroup H. The ratio of haplogroup H in the European populations is less than 5% (Wells et al., 2001; Minárik et al., 2008).

Haplogroup E1b1b (formerly E3b) was the second most frequent haplogroup in the Slovak Romany population. Y-STR analysis identified 16 haplotypes within this haplogroup. Haplogroup E1b1b was found in 42 Romany males (21%) and has a distribution spread from Africa around the Mediterranean into Europe and the Middle East (Cruciani et al., 2004). In the European population, a high frequency of this haplogroup was found in Romania (21.4%), Bulgaria (20.7%), Albania (31.6%), and Italy (11.5%) (Cruciani et al., 2004).

Table 2. Y-STR haplotypes, haplotype frequency (f), predicted Y-haplogroup and probability in Romany population (RP) ($n = 200$) of eastern Slovakia

Haplotype	DYS391	DYS389I	DYS439	DYS389II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385 a/b	n	f(RP)	Predicted Y-haplogroup	Probability (%)
H1	10	12	11	28	10	14	14	11	13	22	15-17	1	0.005	H	99
H2	10	13	11	29	9	14	15	11	12	22	15-17	3	0.15	H	91.1
H3	10	13	11	30	9	14	15	11	12	22	15-17	1	0.005	H	98.5
H4	10	14	10	30	10	16	14	11	13	25	15-16	11	0.055	H	66
H5	10	14	10	30	10	16	14	11	13	25	15-17	7	0.035	H	67.3
H6	10	14	11	28	9	14	15	11	12	22	15-17	1	0.005	H	99.5
H7	10	14	11	28	11	14	15	11	12	22	15-17	1	0.005	H	100
H8	10	14	11	30	9	14	13	11	12	22	15-17	1	0.005	H	97.3
H9	10	14	11	30	9	14	13	12	12	22	15-17	1	0.005	H	96.7
H10	10	14	11	30	9	14	14	11	12	22	15-17	2	0.01	H	95.9
H11	10	14	11	30	9	14	14	11	12	22	15-18	2	0.01	H	91.2
H12	10	14	11	30	9	14	15	11	10	22	15-17	1	0.005	H	94.2
H13	10	14	11	30	9	14	15	11	12	21	15-17	1	0.005	H	99.7
H14	10	14	11	30	9	14	15	11	12	22	15-16	9	0.045	H	99
H15	10	14	11	30	9	14	15	11	12	22	15-17	18	0.09	H	99.1
H16	10	14	11	30	9	14	15	11	12	22	16-16	2	0.01	H	98.3
H17	10	14	11	30	9	14	15	11	12	22	16-17	1	0.005	H	98.6
H18	10	14	11	30	9	14	15	13	12	22	15-17	1	0.005	H	98.6
H19	10	14	11	30	9	14	16	11	12	22	15-16	1	0.005	H	98.3
H20	10	14	11	30	9	16	15	11	12	22	15-17	1	0.005	H	91.8
H21	10	14	11	31	9	14	15	11	12	22	15-17	1	0.005	H	97.5
H22	10	14	12	30	9	14	15	11	12	22	15-17	6	0.03	H	98.3
H23	10	15	10	31	10	16	14	11	13	25	15-16	1	0.005	H	60
H24	10	15	11	30	9	14	15	11	12	22	15-17	1	0.005	H	99.9
H25	10	15	11	31	9	14	15	11	12	22	15-17	1	0.005	H	99.8
H26	10	15	11	31	9	14	15	11	12	23	15-16	1	0.005	H	95.2
H27	11	13	11	29	9	14	14	11	12	22	15-17	1	0.005	H	97.6
H28	11	14	11	30	9	14	15	11	12	22	15-17	2	0.01	H	99.4
H29	9	14	10	30	10	16	14	11	13	25	15-18	1	0.005	E1b1b	91.1
H30	10	12	12	30	10	14	13	11	13	24	16-18	1	0.005	E1b1b	100
H31	10	12	12	31	10	14	13	11	13	24	16-18	1	0.005	E1b1b	100
H32	10	12	13	29	10	14	13	11	12	24	12-18	1	0.005	E1b1b	62.9
H33	10	13	11	31	10	14	13	11	13	24	16-18	2	0.01	E1b1b	100
H34	10	13	12	30	11	14	13	11	12	24	16-18	1	0.005	E1b1b	100
H35	10	13	12	31	10	14	13	11	13	24	16-18	1	0.005	E1b1b	99.9
H36	10	13	12	31	10	14	13	11	13	24	16-18	19	0.095	E1b1b	100
H37	10	13	13	31	10	14	13	11	13	24	16-18	1	0.005	E1b1b	100
H38	10	14	10	30	10	14	14	11	13	25	15-16	1	0.005	E1b1b	83.9
H39	10	14	10	30	10	16	13	11	13	25	15-16	1	0.005	E1b1b	97
H40	10	14	10	30	10	16	14	11	13	25	15-18	5	0.025	E1b1b	82.5
H41	10	14	10	30	10	16	14	11	13	25	16-16	1	0.005	E1b1b	77.4
H42	10	14	10	30	10	16	14	11	13	25	16-18	4	0.02	E1b1b	97.5
H43	10	14	10	31	10	16	14	11	13	25	16-18	1	0.005	E1b1b	99.6
H44	10	14	11	30	10	16	14	11	13	25	16-18	1	0.005	E1b1b	83.8
H45	9	13	11	31	8	13	14	11	12	23	13-16	1	0.005	J2	99
H46	10	12	11	28	9	16	15	11	12	24	14-17	1	0.005	J2	99.8
H47	10	12	12	28	9	16	15	11	12	24	13-13	1	0.005	J2	99.9
H48	10	12	12	28	9	16	15	11	12	24	13-17	1	0.005	J2	99.9

Table 2. (continued)

Haplotype	DYS391	DYS389I	DYS439	DYS389II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385 a/b	n	f(RP)	Predicted Y-haplogroup	Probability (%)
H49	10	13	10	30	9	14	14	11	12	23	13-16	1	0.005	J2	82.9
H50	10	13	11	30	9	14	14	11	12	23	13-16	5	0.25	J2	85.2
H51	10	13	11	30	10	15	14	12	10	24	11-14	1	0.005	J2	88.1
H52	10	13	13	32	9	14	17	11	12	22	13-15	1	0.005	J2	93.4
H53	10	14	10	30	10	16	14	11	12	25	15-16	1	0.005	J2	81.8
H54	10	14	10	30	10	16	14	11	12	25	15-18	1	0.005	J2	76.8
H55	10	14	10	30	10	16	14	11	13	25	14-16	1	0.005	J2	90.3
H56	10	14	11	30	9	14	14	11	12	23	13-16	5	0.025	J2	95.1
H57	10	14	11	30	9	14	14	11	13	23	13-16	1	0.005	J2	93.5
H58	10	14	11	31	9	14	14	11	10	23	13-16	1	0.005	J2	99.9
H59	10	14	11	31	9	14	14	11	12	23	12-15	1	0.005	J2	70.5
H60	10	14	11	31	9	14	14	11	12	23	13-16	7	0.035	J2	91.6
H61	10	14	12	31	9	14	14	11	12	23	13-15	1	0.005	J2	80.4
H62	10	14	13	31	9	14	14	11	12	23	13-16	1	0.005	J2	91.2
H63	11	14	10	31	11	14	16	11	13	26	14-20	1	0.005	J2	100
H64	10	11	12	28	10	16	14	11	13	22	14-14	1	0.005	I1a	98.5
H65	10	12	11	28	10	15	14	11	13	23	13-13	2	0.01	I1a	99.9
H66	10	12	11	28	10	16	14	11	13	22	14-14	1	0.005	I1a	99.9
H67	10	12	11	28	10	16	14	13	13	22	14-14	1	0.005	I1a	99.9
H68	10	12	12	28	10	16	14	11	13	22	14-14	22	0.11	I1a	99.9
H69	10	12	12	28	10	16	14	13	13	22	14-14	1	0.005	I1a	99.9
H70	10	13	12	29	12	14	14	14	13	23	11-14	1	0.005	R1b	99.9
H71	10	13	12	29	12	15	14	13	13	23	11-13	2	0.01	R1b	100
H72	10	13	12	29	12	15	14	13	13	24	11-14	1	0.005	R1b	100
H73	10	13	12	29	12	15	14	13	13	24	14-14	1	0.005	R1b	99.9
H74	11	13	11	29	12	12	14	13	13	24	11-14	1	0.005	R1b	100
H75	11	13	11	30	12	15	14	13	12	24	11-14	2	0.01	R1b	100
H76	12	13	12	29	12	15	14	13	13	25	11-11	1	0.005	R1b	100
H77	10	13	10	29	11	14	17	11	13	24	10-13	1	0.005	R1a	100
H78	10	13	10	30	11	14	15	11	13	24	11-14	1	0.005	R1a	100
H79	10	13	10	30	11	14	16	11	13	24	10-14	1	0.005	R1a	100
H80	10	13	11	30	11	14	16	11	13	24	11-14	2	0.01	R1a	100
H81	11	13	10	30	11	14	17	11	13	25	14-14	1	0.005	R1a	100
H82	10	13	11	30	10	15	16	11	13	24	13-15	1	0.005	I2a	99.9
H83	10	14	10	30	10	14	14	14	14	23	11-13	1	0.005	N1	100

Y-haplogroup predictions for haplotypes where the Bayesian probability was less than 90% (but all were greater than 60%) are shown in red. f (RP), frequency haplotypes in Slovak Romany population.

The third most frequently found haplogroup in the Slovak Romany population was J2 with a frequency of 16.5% (33 Romanies). Haplogroup J2 is presented in different countries such as Turkey, Iraq, Kurdistan, Lebanon, Syria, Armenia, Georgia, Italy, and many Balkan states. Both haplogroups H and J2 have been found in the south and east of India with a frequency ranging from 20 to 30% (Bamshad et al., 2001; Kivisild et al., 2003).

We observed the most frequent haplotype 10-12-12-28-10-16-14-11-13-22-14/14 (DYS391, DYS389I, DYS439, DYS389II, DYS438, DYS437, DYS19, DYS392, DYS393, DYS390, DYS385a/b) within I1a haplogroup in 22 males. Haplogroup I1a was found in 28 individuals (14%). A high frequency of haplogroup I1a has been reported in several Slavic populations (Slovaks, Czechs, Poles, Estonians, Ukrainians, Slovenes, Bosnians, and Macedonians) according to International Society of Genetic Genealogy.

The most prevalent haplogroup in Western Europe is R1b, accounting for almost 70% of all lineages (Campbell, 2007). We found only nine individuals (4.5%) with this haplogroup in the Romany population. The distribution of the next subclade, R1a, shows an increasing west-east frequency and variance gradients with peaks among Finno-Ugric and Slavic speakers (Pericic et al., 2005). Its frequency increases eastwards and reaches a peak in Poland (56.4%), Hungary (60%), and Ukraine (54%) (Semino et al., 2000). We determined six (3%) haplotypes in which haplogroup R1a was predicted. Semino et al. (2000) grouped together more than 95% of European Y chromosomes into 10 phylogenetically distinct haplogroups, where 70–80% of the Y-chromosome gene pool was presented by R1a, R1b, I, and N.

Research into mitochondrial DNA haplogroups presented haplogroup M with a high frequency in the Slovak Romany population and the Romany populations from neighbouring countries (Gresham et al., 2001; Cvjetan et al., 2004; Malyarchuk et al., 2006; Malyarchuk et al., 2008). Haplogroup M is rare in Europe (Richards et al., 1998; Simoni et al., 2000) and is classified as a haplogroup characteristic mainly of Asian populations (Quintana-Murci et al., 1999). Fifteen autosomal STR loci of the eastern Slovak majority population and the Romany population were described (Soták et al., 2008). This study supported the theory that the Romany population was a conglomerate of a genetically isolated founder population and the coexistence of the non-Romany and Romany populations has been influenced by a spontaneous or forced assimilation for many decades and has taken place in spite of the high level of social isolation of Romany populations.

The genetic data about the Slovak Romany population obtained in this study provide significant evidence of their Asian origins, especially Indian origins. Distributions of haplogroups can explain a migration of Romany ancestors from the Indian subcontinent through the Middle East and Balkans to Central Europe. Kalaydjieva's research has shown that the original group appeared in India some 32–40 generations ago and was small, probably under 1000 people (Kalaydjieva et al., 2001a). Consecutive population fissions have occurred after the arrival of the 'gypsies' in Europe, giving rise to numerous sub-isolates.

The achieved results indicated that the Slovak Romany

population lives in socially isolated groups. It is possible to reveal their origin by following their genetic structure. The influences of the genetic drift and different degrees of admixture with non-Romany populations have shaped a peculiar mosaic of paternal lineages of the Slovak Romany population.

Quality control

The laboratory has previously participated in the Y-STR haplotype reference database (<http://www.yhrd.org>) quality assurance exercise in 2008, where five quality control samples were typed correctly with the with Power-Plex Y System.

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