

Romany Population Data on Two Loci (D1S80, Apo BII) from East Slovakia

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ABSTRACT The present paper informs about allele frequency distributions for the two VNTR loci (D1S80, Apo BII) in East Slovak-Romanies. The results are compared with the data of non-Romany Slovak population. Twelve segregating alleles were detected in a total sample of 87 Romany individuals. The most frequent alleles were 37 and 35 on the locus for ApoBII gene. 37/37 occurs as the most frequent genotype in East Slovak-Romanies. 13 alleles were detected in a total sample of 46 Romany individuals on the locus D1S80. 24 and 18 alleles and 24/24 genotype were the most frequent in Romany individuals.

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INTRODUCTION

Throughout the length of the paths of their history, the only documents that Romanies and Travellers have left behind them have for better or worse, factual or fantastic been written by others. Their own collective memory has retained more of the legendary than of certainties. The first Romany groups discovered Europe, in a westward sweep, primarily in the 14th and 15th centuries, and Europe in turn discovered them with a mixture of astonishment, unease, and incomprehension. The groups which arrived in Western Europe often continued their travels from region to region, country to country. Gradually the pace slowed, and the range of travel generally narrowed to the confines of a nation or region. Simultaneously and it seems that this has been so from the very beginning, and in every country some groups have reduced their travels to a minimum, or given them up altogether.

Romany history dates back to the first record of their stay in Europe. Earlier written, historical sources do not exist because the Romanies had no written records or hypotheses about their origin. As a result, various, often mysterious legends came into existence. One of the oldest leg-

ends maintains they came from Egypt. Despite misunderstandings and inaccuracies, some Romany groups misused this legend. Although linguistic studies during the second half of century 18 indicated Indian origin of the Romanies (Rüdiger, 1782; Grellmann, 1787; Pott, 1845), it is not yet known from which part of India they came, to which *jatis* (castes) they belonged and what their social status on the territory of India was. Only few literature data are available to indicate that Romanies belonged to the low paria castes. It is true that at the present time *jatis* (caste) of Dom is "low", but it was not so in history, on contrary, Hindus caste vocabulary Jatikos confirms the political power of Doms in the past and Indian historians reach the agreement they belonged to the original Indian population being of Dravid origin and shared the construction of one oldest civilization of protoindian civilization Mohenjodaro, Harappa, Kalibangan, Lothal etc. (Hübschmannová, 1996). Up to the present time, however, these facts were not published in any study about the Romany history. As no written direct documents about Romanies in India were found, other nonhistorical relevant scientific disciplines, e.g. population genetics and molecular anthropology help to solve this problem.

At the present time a population of about 8 million of Romanies (Gypsies) lives in Europe, including about 400 000 in Slovakia. Efforts leading to the more exact history of the 10 million ethnic group living on our planet has an academic aspect but is also of significant social importance. Information on the ethnocultural and anthropological differentiation of Romanies is limited. Taking into account that history is a scientific branch with an immensely important social output, every ethnic community strives to make use of even the most distant periods in its own history for its social and political aims and

to support its national awareness. One has to consider at this point how far molecular genetics and anthropology may be able to plug the gaps left by history.

MATERIAL AND METHODS

The sample includes a total of 87 unrelated, apparent healthy, male and female Romany subjects from East Slovakia. The tandem repeat polymorphisms used were: D1S80, detected by probe MCT 118, and located on chromosome 1p36-p35 (Kasai et al., 1990), ApoB, the 3'flanking region of the apolipoprotein B gene (Boerwinkle et al., 1989), located on chromosome 2p24-p23. Genomic DNA was purified from blood samples through saturated NaCl (Miller et al., 1988). The PCR reaction was carried out as described by Mullis et al. (1986). Primers used for amplification of VNTR polymorphisms and their origin are together with the thermal profiles and incubation times for both polymorphism in table 1. Each sample that was amplified contained 1 µg of genomic DNA, 2 U of Taq DNA polymerase, 1 µM of each primer, 1.5 mM MgCl₂. The four dNTPs were present each at a final concentration of 200 µM. The total volume of each sample was 50 µl. Each sample was overlaid with 50 µl of mineral oil. The amplification was performed in a Perkin-Elmer Thermocycler according to the conditions given for each locus in the cited references. Fragments generated by PCR were resolved by electrophoresis in 2% agarose gels containing ethidium bromide. After electrophoresis separation of the amplified DNA products the size of the alleles was determined by comparison with an allele markers and 100 bp ladder.

RESULTS AND DISCUSSION

This report shows the application of the polymerase chain reaction to the typing of individual DNAs with two different VNTRs. It indicates the allele distribution of these highly polymorphic DNA markers, determined in a large random sample of individuals. These results represent a further characterization of the Romany population, which we have previously studied for other markers (Bernasovský, 1994; Bernasovský et al., 1994; Bernasovská et al., 1998; etc.)

Allele frequencies for two different tandem repeat DNA loci were determined by PCR of genomic DNA obtained from Romany subjects, after electrophoretic separation of the fragments. Table 2, 3 and figure 1 - 4 summarizes the results obtained for each polymorphic locus in the individuals tested.

ApoBII (Table 2, Fig. 1, 2) - Twelve segregating alleles were detected in a total sample of 87 Romany individuals. The most frequent alleles were 37 (0.4253) and 35 (0.3276). Genotype 37/37 occur as the most frequent genotype in East Slovak-Romanies (0.2644). Allelic designation by Boerwinkle et al. (1989). Our results are generally similar to the data published for these loci in Indian population (Deka et al., 1992). For comparison we selected the data in Slovak-non-Romanies (Kádasi et al., 1994). Some differences were observed, as discussed below, for selected cases. For non-Romanies population (Table 4 and Fig. 1) were found 12 alleles being detected in a total sample of 230 unrelated individuals (Kádasi et al., 1994). Some differences were observed for alleles 39, 47 and 49, their frequencies were lower in Romany

Table 1: PCR primers, thermal profiles and incubation times

VNTR	Sequence of primers used		Reference	
ApoBII	5'-ATGGAAACGGAGAATTATG-3'	5'-CCTTCTCACTTTGGCAATAC-3'	Boerwinkle et al. (1989)	
D1S80 (MCT118)	5'-GAAACTGGCCTCCAAACACTGCCCGCCG-3'	5'-GTCTTGTGGAGATGCACGTGCCCTTGC-3'		
	Denaturation Temp/time	Annealing Temp/time	Polymerization Temp/time	Cycles
ApoBII	95°C/1 min	55°C/1 min	72°C/2 min 72°C/7 min (final extension)	30
D1S80 (MCT118)	94°C/45 sec	64°C/45 sec	70°C/8 min	27

population and further we detected allele 53 but no allele 41. The observed and expected proportion of heterozygotes was 58.62% and 66.68%, ($\chi^2 = 1.66$; $df = 1$). High incident of homozygotes (41.38%) indicates that most Romany communities have been evolved as small and endogamous isolates. Thus, genetic drift, founder effect and inbreeding might have

operated and brought about the genetic differentiation observed nowadays. With the regard of Indian origin of European Romanies to be expected a significant tendency to consanguine marriage in them. Since this tendency is characteristic for a great part of populations living on the Indian subcontinent (Sanghvi, 1966), they have practically the extremely high values of

Table 2: ApoBII-alleles identified in 87 Romanies and their frequencies

Genotype	No. observed (expected)	Frequency	Allele	No. observed	Frequency
29/35	1 (0.32)	0.0115	29	1	0.0057
31/31	1 (0.18)	0.0115	31	8	0.0460
31/33	1 (0.23)	0.0115	33	5	0.0287
31/35	2 (2.62)	0.0230	35	57	0.3276
31/37	2 (3.40)	0.0230	37	74	0.4253
31/39	1 (0.32)	0.0115	39	7	0.0403
33/35	1 (1.64)	0.0115	43	1	0.0057
33/37	2 (2.12)	0.0230	45	4	0.0230
33/51	1 (0.06)	0.0115	47	6	0.0345
35/35	12 (9.34)	0.1379	49	8	0.0460
35/37	16 (24.24)	0.1839	51	2	0.0115
35/39	4 (2.30)	0.0459	53	1	0.0057
35/45	4 (1.31)	0.0459			
35/47	3 (1.97)	0.0345			
35/49	1 (2.62)	0.0115			
35/51	1 (0.66)	0.0115			
37/37	23 (15.73)	0.2644			
37/47	2 (2.55)	0.0230			
37/49	5 (3.40)	0.0575			
37/53	1 (0.42)	0.0115			
39/49	2 (0.32)	0.0230			
43/47	1 (0.03)	0.0115			
Total	87	1.0000		174	1.0000

Table 3: D1S80 (MCT118) - alleles identified in 46 Romanies and their frequencies

Genotype	No. observed (expected)	Frequency	Allele	No. observed	Frequency
16/18	1 (0.50)	0.0217	16	2	0.0217
16/22	1 (0.06)	0.0217	18	23	0.2500
18/18	6 (2.88)	0.1304	21	2	0.0217
18/23	1 (0.50)	0.0217	22	3	0.0326
18/24	9 (11.24)	0.1958	23	2	0.0217
21/23	1 (0.04)	0.0217	24	45	0.4891
21/31	1 (0.07)	0.0217	25	3	0.0326
22/24	1 (1.47)	0.0217	26	4	0.0435
22/36	1 (0.03)	0.0217	27	2	0.0217
24/24	12 (11.00)	0.2609	28	1	0.0109
24/25	3 (1.47)	0.0653	31	3	0.0326
24/26	4 (1.96)	0.0870	35	1	0.0109
24/27	2 (0.97)	0.0436	36	1	0.0109
24/31	1 (1.47)	0.0217			
24/35	1 (0.49)	0.0217			
28/31	1 (0.03)	0.0217			
Total	46	1.0000		92	1.0000

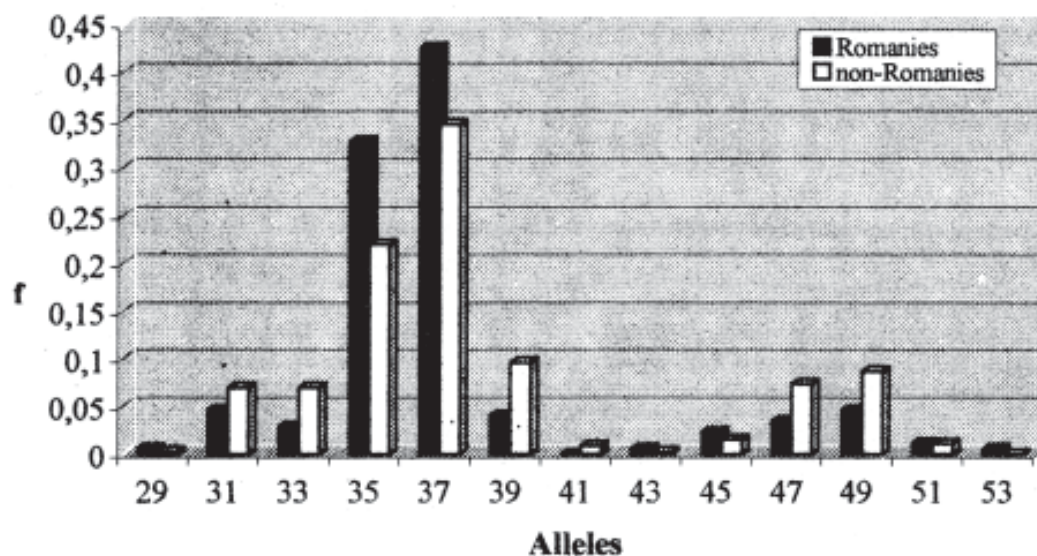


Fig. 1. ApoBII - Comparison of identified alleles and their frequencies in Romanies from East Slovakia and non-Romanies from Slovakia (Kádasi et al., 1994)

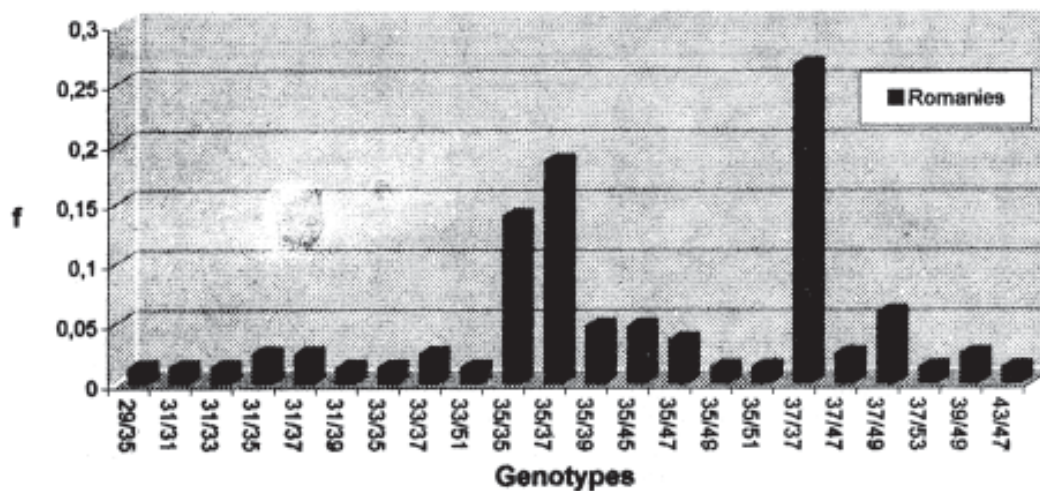


Fig. 2. ApoBII - Genotypes and their frequencies in Romanies from East Slovakia

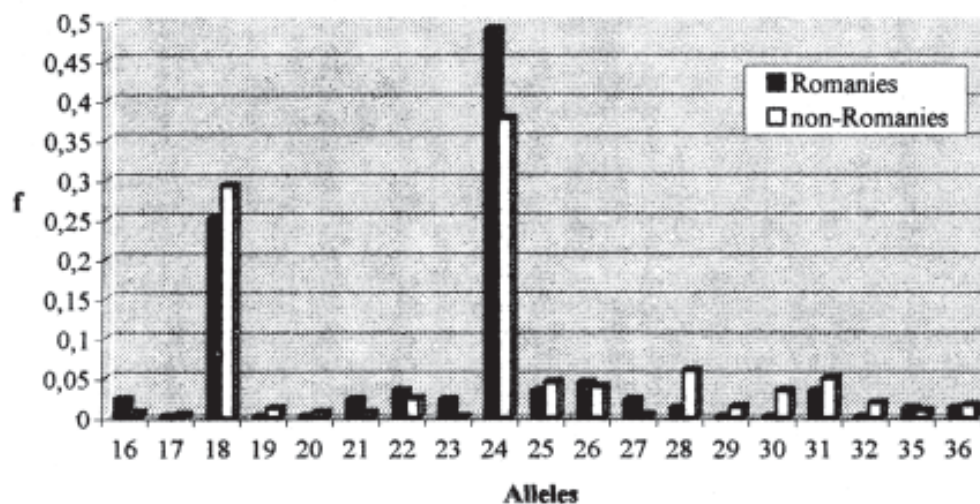


Fig. 3. MCT 118 (D1S80) - Comparison of identified alleles and their frequencies in Romanies from East Slovakia and non-Romanies from Slovakia (Kádasi et al., 1994)

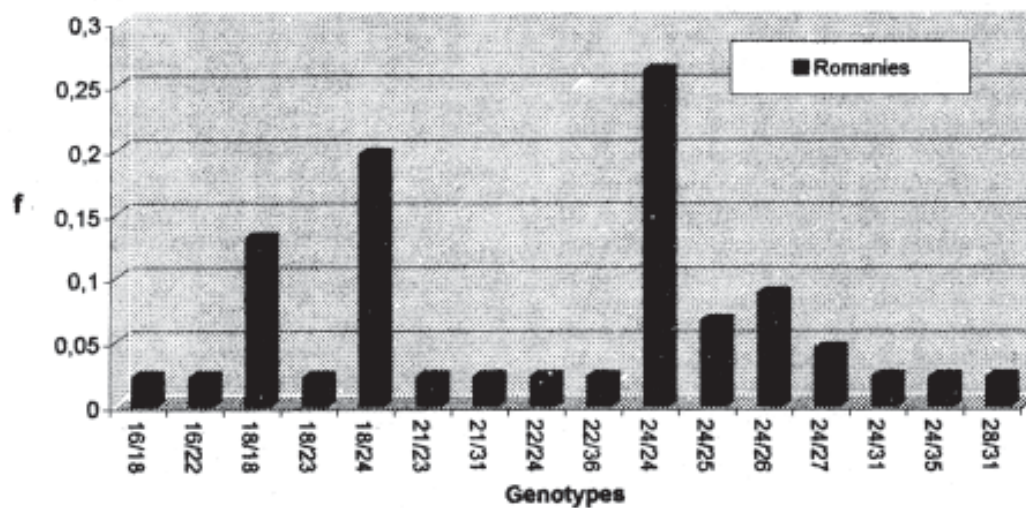


Fig. 4. MCT118 (D1S80) - Genotypes and their frequencies in Romanies from East Slovakia

Table 4: ApoBII and D1S80 (MCT118) - alleles identified in 230 unrelated Slovaks and estimate of their frequencies (Kádasi et al., 1994)

<i>ApoBII</i>	<i>Allele</i>	<i>No. observed</i>	<i>Frequency</i>	<i>MCT118</i>	<i>Allele</i>	<i>No. observed</i>	<i>Frequency</i>
	29	1	0.0022		16	2	0.0051
	31	32	0.0696		17	1	0.0026
	33	32	0.0696		18	114	0.2923
	35	101	0.2196		19	4	0.0103
	37	159	0.3457		20	2	0.0051
	39	44	0.0957		21	2	0.0051
	41	4	0.0087		22	9	0.0231
	43	1	0.0022		24	147	0.3769
	45	7	0.0153		25	17	0.0436
	47	34	0.0739		26	15	0.0385
	49	40	0.0870		27	1	0.0026
	50	5	0.0109		28	23	0.0590
					29	5	0.0128
					30	13	0.0333
					31	19	0.0487
					32	7	0.0180
					35	3	0.0077
					36	6	0.0154
Total		460	1.0000			390	1.0000

inbreeding coefficient. The marriage customs are known as most stable and conservative features in the Indian society.

D1S80 (MCT118) - Out of alleles described so far, 13 were detected in a total sample of 46 Romany individuals. Each allele was completely resolved based on increments of the repeat unit of the VNTR locus. The length of the repeat unit has been determined to be 16 base pairs (Nakamura et al., 1988). Two of them, alleles 24 (0.4891) and 18 (0.2500) clearly predominate. 24/24 occur as the most frequent genotype in East Slovakian Romanies with frequency 0.2609. The share of heterozygotes was 60.87%. The distribution of the phenotypes is in Hardy-Weinberg equilibrium ($\chi^2 = 3.08$; $df = 1$). The observed and expected genotypes and alleles are given on table 3 and figure 3, 4. The frequencies found in our Romany series were compared with frequencies in Slovakia non-Romany population (Kádasi et al., 1994). Our results resemble the data published for this loci in Slovak non-Romany population (Table 4 and Fig. 3). As seen, no alleles 17, 19, 20, 29, 30, 32, 33 and 34 were detected and the frequency of alleles 24, 16, 21 and 27 was higher in our samples.

According to the data of population genetic investigation in Romanies result that the gene pool of the present-day Romany population in

Europe is significantly different from the majority of European population.

REFERENCES

- Bernasovská, J., Bernasovský, I., Barlová, E., Chlebovská, K. and Biroš, E.: Serogenetic study of blood and serum groups, red cell isoenzymes, HLA antigens of first class and apolipoprotein B gene polymorphism. *J. Hum. Ecol.*, **9**(2): 113-121 (1998).
- Bernasovský, I.: Seroanthropology of Roms (Gypsies). Moravian Museum, Brno. PP. 76 (1994).
- Bernasovský, I., Juričková, J. and Ferák, V.: Population genetic study in Gypsies (Roms) from Slovakia: Distribution of blood group genetic markers. *Anthropol. Sci., Tokyo*, **102**(4): 409-419 (1994).
- Boerwinkle, E., Xiong, W., Fourest, E. and Chang, L.: Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: Application to the apolipoprotein B3' hypervariable region. *Proc. Natl. Acad. Sci. USA*, **86**: 212-216 (1989).
- Deka, R., Chakraborty, R., DeCroo, S., Rothhammer, F., Barton, S.A. and Ferrell, E.: Characteristics of polymorphism at a VNTR locus 3' to the apolipoprotein B gene in five human populations. *Am. J. Hum. Genet.*, **51**: 1325-1333 (1992).
- Grellmann, H.M.G.: *Historischer Versuch über die Zigeuner betreffend die Lebensart und Verfassung, Sitten und Schicksale dieses Volks seit seiner Erscheinung in Europa und dessen Ursprung*. Göttingen (1787).
- Hübschmannová, M.: Personal communication (1996).
- Kádasi, L., Géczi, J., Feráková, I., Lubyová, B., Bohušová, T., Feráková, E. and Paláková, H.: Distribution of ApoBII, MCT118 (D1S80), YNZ22 (D17S30), and Col2A1 AmpFLPs (Amplified Fragment Length Polymorphisms) in Caucasoid population of Slovakia. *Gene Geography*, **8**: 121-127 (1994).

- Kasai, K., Nakamura, Y. and White, R.: Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by polymerase chain reaction (PCR) and its application to forensic science. *J. Foren. Sci.*, **35**: 1196-1200 (1990).
- Miller, S.A., Dukes, D.D. and Polesky, H.F.: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **16**: 1215 (1988).
- Mullis, K.B., Faloona, F.A., Scharf, S.J., Saiki, R.K., Horn, G.T. and Erlich, H.A.: Specific enzymatic amplification of DNA *in vitro*: The polymerase chain reaction. *Cold Spring Harbor Symp. Quant Biol.*, **51**: 263-273 (1986).
- Nakamura, Y., Carlson, M., Krapcho, V. and White, R.: Isolation and mapping of a polymorphic DNA sequence (pMCT118) on chromosome 1 p (D1S80). *Nucleic Acids Res.*, **16**: 9364 (1988).
- Poti, A.F.: *Die Zigeuner in Europa und Asien*. Halle (1845).
- Rüdiger, Ch.: *Von der Sprachen und Herkunft der Zigeuner aus Indien*. Leipzig, Halle (1782).
- Sanghvi, L.D.: Inbreeding in India. *Eugen Quart.*, **13**: (1966).