ELSEVIER

Forensic Science International 124 (2001) 182–186

Forensic Science International

www.elsevier.com/locate/forsciint

Y-chromosomal STR haplotypes in three major population groups in Bulgaria

Boriana Zaharova[a,*], Silvia Andonova[a], Anja Gilissen[b], Jean-Jacques Cassiman[b], Ronny Decorte[b], Ivo Kremensky[a]

[a]*Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynaecology, 2 Zdrave St, 1431 Sofia, Bulgaria*
[b]*Laboratory for Forensic Genetics and Molecular Archaeology, Center for Human Genetics, K.U. Leuven, Belgium*

Received 6 April 2001; received in revised form 18 September 2001; accepted 19 September 2001

Abstract

A total of 280 unrelated males from the three largest population groups in Bulgaria: Bulgarians, Bulgarian Turks and Gypsies, were analyzed for seven Y-chromosome STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393). Comparison of the allele frequency distributions revealed significant differences between the three ethnic groups which were confirmed with haplotype analysis. This permits us to suggest that population differentiation should be taken into account in forensic case analysis and paternity testing in Bulgaria. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Y-chromosome STRs; Y-haplotypes; Forensic genetics; Population genetics

Populations: Unrelated healthy males (127 Bulgarians, 66 Bulgarian Turks, 91 Gypsies) from the Bulgarian DNA bank and fathers from routine paternity cases born in various geographical regions of Bulgaria.

Extraction: Genomic DNA was extracted from 38 blood stains (Guthrie cards) with the QIAamp Tissue Kit (QIAGEN GmbH, Germany) and further concentrated on MICROCON100 devices (Amicon, USA). For venous blood samples saline extraction and for saliva swabs phenol–chloroform extraction were used.

PCR: Triplex DYS390, DYS391, DYS393 and singleplex amplification of DYS19 and DYS392 were performed as described elsewhere [1]. PCR of DYS389 I/II loci was carried out in a volume of 12.5 μl containing 150 ng of DNA, 0.56 μM of each primer, 1× reaction buffer (2.25 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 50 mM Tris.HCl, pH 8.2 (20 °C), 0.01 mM EDTA, 2% (v/v) DMSO, 0.1% Tween 20), 200 μM dNTPs and 0.75 U STS polymerase (Scientific Technological Service, Sofia). The cycling conditions were: initial denaturation for 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 45 s at 65 °C, 60 s at 72 °C and final extension for 5 min at 72 °C.

Typing: Diluted PCR-products were mixed with 106/347 bp or 106/463 bp internal size standards [2] and analyzed on an ALF Express DNA sequencer (Amersham Pharmacia Biotech) in comparison with sequenced allelic ladders, using 6% 19:1 acrylamide: bisacrylamide 7 M Urea gels for 90–220 min at 2000 V, 70 mA, 45 W, 50 °C. The nomenclature in the Y-STR Haplotype Reference Database (http://ystr.charite.de) was applied for all loci except DYS389II. The latter was analyzed after subtraction of DYS389I alleles and the allelic designation of Kayser et al. was used [3].

Results: See Table 1.

Analysis of data: Haplotype diversity values were calculated as $D = (n/(n-1))(1-\sum p_i^2)$, where p_i is the frequency of the ith haplotype and n is the number of analyzed individuals [4]; exact test for population differentiation was performed using GENEPOP V3.2 [5].

Disponibility of the data: zaharova@ns.medfac.acad.bg.

Other remarks: The analysis of seven Y-chromosome STRs revealed a heterogeneous male structure of the Bulgarian population and demonstrated significant differences among the three largest ethnic groups in Bulgaria. The Gypsy allele frequency distributions in all studied loci

* Corresponding author. Tel.: +359-2-95-20-490; fax: +359-2-95-20-124.
E-mail address: zaharova@ns.medfac.acad.bg (B. Zaharova).

PII: S0379-0738(01)00597-7

Table 1
Y-chromosomal STR haplotypes in the three Bulgarian population groups: B-Bulgarians ($n = 126$), BT-Bulgarian Turks ($n = 63$), G-Gypsies ($n = 91$)[a]

No.	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	B	BT	G
h1	12	14	19	24	9	11	14		1	
h2	13	12	16	22	10	11	12		1	
h3	13	12	16	22	10	11	13		1	
h4	13	12	16	22	10	15	13		1	
h5	13	12	16	24	10	11	12	1		
h6	13	12	17	24	10	11	13	1		
h7	13	12	18	24	10	11	13			1
h8	13	13	15	23	10	13	13	1		
h9	13	13	16	22	10	15	13	2		
h10	13	13	16	24	10	11	13	3		
h11	13	13	16	24	11	11	13	1		
h12	13	13	17	22	10	16	11	1		
h13	13	13	17	24	10	11	12		1	
h14	13	13	17	24	10	11	13	12	3	2
h15	13	13	17	24	10	12	13	1		
h16	13	13	17	24	11	11	13	2		
h17	13	13	17	25	10	11	13	2		1
h18	13	13	18	24	11	11	13	1		1
h19	13	13	18	24	9	11	14			1
h20	13	13	19	25	10	11	13			3
h21	13	14	16	22	10	15	13	2	1	
h22	13	14	17	24	10	11	13	2	1	
h23	13	14	19	24	9	11	13			1
h24	14	12	16	22	10	11	13	1	1	8
h25	14	12	16	22	10	11	15	1		
h26	14	12	16	23	10	11	13		1	
h27	14	12	16	23	10	12	12		1	
h28	14	12	16	24	10	12	13			1
h29	14	12	16	24	11	13	13	1		
h30	14	12	17	22	10	11	13	1		
h31	14	12	17	23	10	11	13	1		
h32	14	12	19	23	10	11	14		1	
h33	14	13	15	25	11	13	13		1	
h34	14	13	16	22	10	11	12		1	
h35	14	13	16	22	10	11	13			1
h36	14	13	16	22	11	11	12		1	
h37	14	13	16	22	9	11	12	1		
h38	14	13	16	23	10	13	13	1	1	
h39	14	13	16	23	10	14	13		1	
h40	14	13	16	23	11	11	12	1		
h41	14	13	16	24	11	12	12	1		
h42	14	13	16	24	11	13	12	2	1	
h43	14	13	16	24	11	13	13	2	1	
h44	14	13	16	24	11	13	14	1		
h45	14	13	16	25	11	13	12	1		
h46	14	13	17	22	10	11	12	1		
h47	14	13	17	22	10	15	12		1	
h48	14	13	17	23	10	11	12		2	
h49	14	13	17	23	11	11	12	1		
h50	14	13	17	24	10	11	12			1
h51	14	13	17	24	10	11	13		2	
h52	14	13	17	26	11	13	12		1	
h53	14	13	18	22	10	11	13			1
h54	14	13	18	23	10	11	12	1	1	
h55	14	14	15	23	10	14	12	1		
h56	14	14	16	22	10	11	12			5

Table 1 (*Continued*)

No.	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	B	BT	G
h57	14	14	16	23	10	11	12		1	
h58	14	14	16	23	11	14	14		1	
h59	14	14	16	24	10	11	16			1
h60	14	14	16	25	10	11	13			1
h61	14	14	16	25	11	13	12	1	1	
h62	14	14	17	22	10	11	12		1	
h63	14	14	17	23	10	11	12	1		
h64	14	14	17	23	10	11	13	1		
h65	14	14	18	23	11	11	13			1
h66	14	14	18	25	10	11	13		1	
h67	15	12	15	24	10	11	12			1
h68	15	12	16	21	10	11	14		1	
h69	15	12	16	22	10	11	13			1
h70	15	12	16	22	10	12	13	1		
h71	15	12	16	22	11	11	14		1	
h72	15	12	16	23	10	11	12		1	
h73	15	12	16	24	10	11	12	2		
h74	15	12	16	24	10	11	13	2		
h75	15	12	16	24	10	12	12	1		
h76	15	12	16	24	11	11	12		1	
h77	15	12	16	25	11	11	12		1	
h78	15	12	17	21	11	11	15	1		
h79	15	12	17	24	10	11	12		2	
h80	15	12	18	21	10	11	14	2		
h81	15	12	18	22	10	10	14		1	
h82	15	12	18	22	10	11	13	1		
h83	15	12	18	24	10	12	14		1	
h84	15	13	16	22	10	11	12	1		3
h85	15	13	16	22	11	13	13	1		
h86	15	13	16	23	10	11	12	1	1	3
h87	15	13	16	23	10	13	13	1		
h88	15	13	16	23	9	11	12	2		
h89	15	13	16	23	9	12	12	1		
h90	15	13	16	24	10	12	14	1		
h91	15	13	16	24	10	12	15	1		
h92	15	13	16	24	11	11	13	1		
h93	15	13	16	24	11	13	12	1		
h94	15	13	16	25	10	11	13	1		1
h95	15	13	16	25	11	13	13	1		
h96	15	13	17	22	10	11	12	1		3
h97	15	13	17	22	10	11	13	1		
h98	15	13	17	23	10	11	12	1	1	
h99	15	13	17	24	10	11	14			1
h100	15	13	17	24	11	11	13	2	1	
h101	15	13	17	24	11	13	13	1		
h102	15	13	17	25	10	11	13	2		
h103	15	13	17	25	11	11	13	1		
h104	15	13	17	25	9	11	12	1		
h105	15	13	18	23	10	11	12	1		
h106	15	13	18	23	11	11	13	1		
h107	15	13	18	24	11	11	13	2		
h108	15	13	18	25	11	11	13			3
h109	15	13	20	24	11	11	13	1		
h110	15	14	16	22	10	11	12		4	24
h111	15	14	16	22	11	11	12			1
h112	15	14	16	24	10	11	12	1		
h113	15	14	17	22	10	11	12		1	
h114	15	14	17	22	11	11	16	1		

Table 1 (*Continued*)

No.	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	B	BT	G
h115	15	14	19	25	11	10	13	1		
h116	15	15	16	22	10	11	12			1
h117	16	13	15	25	10	11	13	1		
h118	16	13	16	22	10	11	14			1
h119	16	13	16	23	9	11	12	1		
h120	16	13	16	24	10	11	14	1		
h121	16	13	16	25	10	11	12		1	
h122	16	13	16	25	11	11	13	1		
h123	16	13	17	22	11	11	13		1	
h124	16	13	17	24	10	11	13	2		
h125	16	13	17	24	10	11	14	1		
h126	16	13	17	24	11	11	13	2		
h127	16	13	17	25	10	11	13		1	
h128	16	13	17	25	11	11	13	1	1	1
h129	16	13	18	22	10	11	12			1
h130	16	13	18	22	11	11	13	1		
h131	16	13	18	23	10	11	12			1
h132	16	13	18	23	10	11	13	1		
h133	16	13	18	24	10	11	13	3	1	
h134	16	13	18	24	11	11	13	4	2	1
h135	16	13	18	25	10	11	13			1
h136	16	13	18	25	11	11	13		1	
h137	16	13	19	22	10	11	12	1		3
h138	16	13	19	23	11	11	13	1		
h139	16	13	19	24	10	11	13	3		
h140	16	13	19	24	11	11	13	2		
h141	16	13	19	24	11	11	14	1		
h142	16	13	19	25	10	11	13			1
h143	16	14	16	22	10	11	12		1	1
h144	16	14	16	25	10	11	13			1
h145	16	14	16	25	10	13	12			1
h146	16	14	16	25	11	11	13		1	
h147	16	14	17	25	10	11	13	2		
h148	16	14	18	25	11	11	13		1	
h149	16	14	19	22	10	11	14	1		
h150	17	13	17	24	10	11	13		1	5
h151	17	13	17	24	10	11	14	1		
h152	17	14	16	24	10	11	13			1
h153	18	13	17	25	11	11	13		1	

[a] Haplotype diversity/discrimination index: B—0.987; BT—0.993; G—0.916.

strongly differed from the Bulgarian ($P = 0.00000$) and Turkish ($P = 0.00000$–0.00457) as well as four loci (DYS19, DYS389I, DYS390 and DYS393) showed significant differences between the groups of Bulgarians and Bulgarian Turks ($P = 0.00000$–0.00018). The haplotype analysis revealed a high number (129) of population-specific haplotypes (sampled only in a particular population group) as well as a relatively low degree of haplotype sharing between the three groups. In addition, different founder haplotypes in the groups of Bulgarians and Gypsies have been detected [1]. The observed most common haplotype in Gypsies (h110 in Table 1; frequency 26.4%) probably corresponds to the microsatellite haplotype 3 which predominate in the ''Vlax Roma'' founder haplogroup 35, reported by Kalaydjieva et al. [6]. This haplotype was not present in the Bulgarian sample, but appeared also in the Turkish group at a considerably lower frequency (6.3%). Bulgarian most common haplotype (h14 in Table 1; frequency 9.5%) was found also in three Turkish and two Gypsy males. The observed differences among the Bulgarian population groups permit us to suggest that population differentiation should be taken into account in forensic case analysis and paternity testing in Bulgaria. The studied set of seven Y-chromosome STRs demonstrated a considerably higher power to discriminate between males within Bulgarian and Turkish groups (99%) and even between individuals in the genetically isolated Gypsy group (92%) than reported for other European populations (between 74% and 90%) [7].

Acknowledgements

We thank Dr. P. de Knijff (Leiden University) for providing us allelic ladders and control samples for the investigated STR loci. This work was supported by a TEMPUS grant (Project JEP No. 081-21-94/2; to BZ), a grant from the Fund for Scientific Research, Flanders (no. G.0241.98; to RD) and a grant from the National Science Research Council of Bulgarian Ministry of Education and Science (Project L 907/99).

References

[1] B. Zaharova, A. Gilissen, S. Andonova-Baklova, J.-J. Cassiman, R. Decorte, I. Kremensky, Network analysis of Y-chromosome compound haplotypes in three Bulgarian population groups, in: C. Renfrew, K. Boyle (Eds.), Archaeogenetics: DNA and the Population Prehistory of Europe, McDonald Institute for Archaeological Research, Cambridge, 2000, pp. 237–243.

[2] R. Decorte, J.-J. Cassiman, Evaluation of the ALF sequencer for high-speed sizing of short tandem repeat alleles, Electrophoresis 17 (1996) 1542–1549.

[3] M. Kayser, L. Roewer, M. Hedman, L. Henke, J. Henke, S. Brauer, C. Kruger, M. Krawczak, M. Nagy, T. Dobosz, R. Szibor, P. de Knijff, M. Stoneking, A. Sajantila, Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs, Am. J. Hum. Genet. 66 (2000) 1580–1588.

[4] M. Nei, in: Molecular Evolutionary Genetics, Columbia University Press, New York, 1987, p. 179.

[5] M. Raymond, F. Rousset, GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism, J. Heredity 86 (1995) 248–249.

[6] L. Kalaydjieva, F. Calafell, M. Jobling, D. Angelicheva, P. de Knijff, Z. Rosser, M. Hurles, P. Underhill, I. Tournev, E. Marushiako, Ea.V. Popov, Patterns of inter- and intra-group genetic diversity in the Vlax Roma as revealed by Y-chromosome and mitochondrial DNA lineages, Eur. J. Hum. Genet. 9 (2001) 97–104.

[7] M. Kayser, A. Caglia, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya, W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccinini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G. Weichhold, P. de Knijff, L. Roewer, Evaluation of Y-chromosomal STRs: a multicenter study, Int. J. Legal Med. 110 (1997) 125–133.