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SHORT COMMUNICATION

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Hungarian population data of four X-linked markers: DXS8378, DXS7132, HPRTB, and DXS7423

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Abstract DXS8378, DXS7132, HPRTB, and DXS7423 microsatellite markers located in four different X-chromosomal linkage groups were studied in the Hungarian population. After genotyping unrelated men (219) and women (165), forensic efficiency parameters showing that the four X-linked short tandem repeats are informative for forensic applications were calculated. With fragment and sequence analysis, one microvariant allele (11.2) was identified in the HPRTB locus. A deviation from the Hardy–Weinberg equilibrium could not be detected. Investigations of 96 father–child meioses revealed one mutation in the DXS7132 locus. For comparison of 22 different populations, *G*-tests were carried out.

Keywords X-chromosome · STRs · Deficiency paternity testing · Mutation · *G*-statistics

Introduction

Fathers transmit their X-chromosome to daughters as haplotypes. Analysis of X-chromosomal loci might be beneficial in deficiency paternity cases, where half-sisters and/or grandmothers are examined [20]. The commercially available Mentype Argus X-UL Kit (Biotype AG, Dresden, Germany) makes it possible to examine the DXS8378, DXS7132, HPRTB, and DXS7423 loci belonging to four different linkage groups in one multiplex reaction. The four X-chromosomal loci have been previously studied in other populations: the DXS8378 locus in German [6] and Korean [18] populations; the DXS7132 locus in Taiwanese [4], German [6], and Korean [18] populations; the HPRTB locus in Brazilian [1, 12], Taiwanese [5], German [6], American (Caucasian, African, Mexican, and Asian) [7], Hungarian [8], Basquian (Spain) [9], Japanese [10], Polish [11], Belgian [13], British (UK) [14], Tamilian [15], Mexican [16], Argentinian [17], Korean [18, 19], Tuscanian [21], and Chinese [22] populations; and the DXS7423 locus in African (Casablanca) [3], German [6], Cantabrian [23], and Korean [18] populations.

This paper aims continue the process of establishing a suitable X-chromosomal short tandem repeat (X-STR) database for four loci in the Hungarian population, mainly for complementary testing in deficiency paternity cases with autosomal DNA markers [2, 20].

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Materials and methods

Independently of regions in Hungary, peripheral blood was collected from unrelated men (219), women (165), and families (96) (father, mother, and daughter). Genomic DNA was isolated from blood samples with the QIAmp Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Genomic DNA was amplified using the Mentype Argus X-UL Kit (Biotype AG). PCR products were analyzed by capillary electrophoresis using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment sizes were determined using the GeneScan 3.1.2 software, and allele designations were performed using the Argus X-UL 310_v0.gta Macro software (Biotype AG).

An HPRTB 11.2 allele from a female sample was cloned into the plasmid pCR2.1-TOPO with the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and was transformed into *Escherichia coli* TOP10F′ (Invitrogen). Sequencing reaction of the HPRTB 11.2 allele was performed using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and was analyzed by the ABI Prism 310 Genetic Analyzer capillary electrophoresis system. Primer sequences for amplification of the HPRTB locus were F-5′-

Table 1 Allele frequencies for X-STR markers in the Hungarian population with testing of 165 women and 219 men

Allele	DXS8378	HPRTB	DXS7423	DXS7132	Combined
6	–	–	–	0.002	
7	–	–	–	–	
8	–	0.004	–	–	
9	0.024	0.022	–	–	
10	0.315	0.005	–	–	
11	0.366	0.113	–	0.002	
11.2	–	0.002	–	–	
12	0.251	0.322	0.002	0.122	
13	0.031	0.324	0.135	0.262	
14	0.011	0.158	0.342	0.359	
15	0.002	0.044	0.368	0.193	
16	–	0.005	0.131	0.051	
17	–	–	0.018	0.007	
18	–	–	–	–	
19	–	–	0.002	0.002	
PIC	0.645	0.711	0.659	0.707	NA
PD^{female}	0.852	0.908	0.873	0.900	0.9998
PD^{male}	0.704	0.719	0.694	0.734	0.9932
HET^{exp}	0.702	0.751	0.710	0.748	NA
HET^{obs}	0.745	0.733	0.776	0.794	NA
$MEC^{Krüger}$	0.440	0.528	0.462	0.520	0.9318
$MEC^{Kishida}$	0.645	0.711	0.659	0.707	0.9898
P values for the HWE	0.064	0.152	0.869	0.477	NA

PIC polymorphism information content, *PD* power of discrimination, HET^{exp} expected heterozygosis, HET^{obs} observed heterozygosis, $MEC^{Krüger}$ mean exclusion chance for deficiency cases (mother, daughter, and putative grandmother), $MEC^{Kishida}$ mean exclusion chance for normal trios (mother, daughter, and putative father), *NA* not applicable, *HWE* Hardy–Weinberg equilibrium

AACTTTTTCTCTGTATGTAGTCGATT-3′ and R-5′-CT ACATCAAAGTGAAAAGCCAT-3′. For sequencing, M13 primers were used.

G-tests were performed with the PopTools software (http://www.cse.csiro.au/poptools.html). The Hardy–Weinberg equilibrium (HWE) was tested in female samples with the Arlequin software (http://lgb.unige.

Table 2 Comparison of the Hungarian population data with other populations using *G*-statistics (*P* values)

Populations	DXS8378	HPRTB	DXS7423	DXS7132
Hungarian [8]	ND	0.927033	ND	ND
Polish [11][a]	ND	0.865041	ND	ND
Belgian [13]	ND	0.612909	ND	ND
Brazilian [1, 12][b]	ND	0.46536	ND	ND
Caucasian-American [7][a]	ND	0.294851	ND	ND
Mexican-American [7][a]	ND	0.071834	ND	ND
German [6]	0.126128	0.052073	0.010024	0.189032
Asian-American [7][a]	ND	0.044168	ND	ND
British (UK) [14]	ND	0.024586	ND	ND
Mexican [16][a]	ND	0.019356	ND	ND
African-American [7][a]	ND	0.015876	ND	ND
Argentinian [17][b]	ND	0.011268	ND	ND
Tamilian (India) [15][a]	ND	0.000578	ND	ND
Basquian (Spain) [9]	ND	0.000339	ND	ND
Taiwanese [4, 5]	ND	0.00014	ND	0.077355
Korean [19]	ND	9.50×10^{-7}	ND	ND
Japanese [10]	ND	1.70×10^{-7}	ND	ND
Korean [18]	0	2.00×10^{-8}	0	0.4235
Tuscanian (Italy) [21][a]	ND	4.41×10^{-16}	ND	ND
Chinese [22][a,b]	ND	0	ND	ND
Cantabrian (Spain) [23][a]	ND	ND	0.035242	ND
African (Casablanca) [3]	ND	ND	1.35×10^{-5}	ND

The difference between the two populations is significant at $P<0.05$
ND no data
[a]The population was less than 200 persons
[b]Populations were pooled together

ch/arlequin.html). Polymorphism information content (PIC), power of discrimination (PD), and mean exclusion chance (MEC) were calculated as before [20].

Results and discussion

With genotyping of 165 women and 219 men, allele frequencies and forensic efficiency parameters (PIC, PD^{male}, PD^{female}, HET^{exp}, HET^{obs}, $MEC^{Krüger}$, $MEC^{Kishida}$, and *P* values for the HWE) of the four X-STR loci were determined in the Hungarian population (Table 1). Based on the combined PD and MEC values, the unlinked X-STR loci provided the possibility for the investigation of paternity in normal and deficiency cases, where the alleged grandmother is examined instead of the alleged father [20]. The calculation of likelihood with unlinked X-STR markers (putative grandmother to daughter) can be carried out as with autosomal markers (putative father to daughter). The combined PD value was 0.9932 for men and was 0.9998 for women. For examination of normal trios, the combined $MEC^{Kishida}$ value was 0.9898; for deficiency cases, the combined $MEC^{Krüger}$ value was 0.9318. Deviations from the HWE could not be detected. Investigations of 96 father–child meioses revealed one mutation in the DXS7132 locus. Testing the same 96 families for nine autosomal markers (ProfilerPlus; Applied Biosystems) showed that the alleged father was the biological father of the child in all cases. There was one microvariant allele (11.2) determined in the HPRTB locus. Sequence analyses revealed an AG deletion at the 3′ end of the flanking region, 48 base pairs from the last repeat. The same allele was found before in a male sample in the Hungarian population [8].

For comparisons of 22 different populations, *G*-tests were carried out (Table 2). Allele distribution between two populations was considered significantly different at $P<0.05$. There were significant differences detected between the Hungarian and the Asian, African-American, African (Casablanca), Mexican, and Argentinian populations in all cases, except for the Taiwanese and Korean populations concerning the DXS7132 locus. Comparisons of the European populations with the Hungarian population revealed no significant differences—except when DXS7423 loci in the German and Cantabrian (Spain) populations were compared, and when HPRTB loci in the British (UK), Basquian (Spain), and Tuscanian (Italy) populations were compared.

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