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SHORT COMMUNICATION

An X-STR meiosis study in Kurds and Germans: allele frequencies and mutation rates

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Abstract X-linked short tandem repeats (X-STRs) play an important supplementary role in the field of forensic genetics, especially in deficiency cases. This paper presents population genetic data for the microsatellite markers DXS8378, DXS6800, DXS101, HPRTB, and DXS8377 in a German and a Kurdish population sample. Buccal swabs were obtained from 217 unrelated healthy German individuals (107 women and 110 men) from the area of Münster and 208 unrelated Kurdish individuals (103 women and 105 men), immigrants mainly from Northern Iraq. Additionally, more than 1,200 meiotic transfers (419 paternal and 819 maternal meioses) were investigated in the systems DXS6800, DXS101, and DXS8377. Five mutations were found in the system DXS8377. With the power of discrimination in females [PD(F)] ranging from 0.81 (DXS8378 in Kurds) to 0.99 (DXS8377 in Germans), the investigated X-STRs systems turned out to be highly informative in the two populations.

Keywords Microsatellites · Kurds · Population genetics · Multiplex PCR · Mutations

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Introduction

X-linked short tandem repeats (X-STRs) can offer several advantages in paternity and maternity cases either with complex deficiencies or questionable mother/son relationship [1, 2].

The simultaneous amplification of X-STRs has been recently reported by other laboratories [3, 4] because of its practical and economical advantages.

In the following, we have applied five X-STRs known to be highly informative in several populations and covering all four linkage groups [2]. In addition to allele frequencies, we also present mutational data. This is the first time that these markers have been characterized in a Kurdish population, while data for German populations from different regions have already been published [5–9].

Materials and methods

Buccal swabs were obtained from 217 unrelated healthy German individuals (107 women and 110 men) from the area of Münster [10] and 208 unrelated Kurdish individuals (103 women and 105 men), mainly immigrants from Northern Iraq [11]. Mutational data were obtained from 80 German (43 women, 37 men) and 244 Kurdish (132 women, 112 men) offspring.

A Chelex extraction from oral cotton swabs was performed as described previously [12]. Multiplex amplification was performed in a pentaplex reaction as described by Tabbada et al. [9] using DXS8378 instead of DXS6789 and in a triplex reaction described by Wiegand et al. [8]. Amplification was performed in a 25-μl volume containing

Table 1 Forensic efficiency parameters

Locus	DXS8378	DXS6800	DXS101	HPRTB	DXS8377
Germans					
Alleles	7	7	15	9	19
MEC	0.42	0.47	0.76	0.51	0.84
PIC	0.63	0.66	0.87	0.69	0.92
HET±SE	0.69±0.03	0.71±0.03	0.44±0.02	0.74±0.03	0.92±0.02
PD(F)	0.85	0.87	0.98	0.89	0.99
p(HWE)	0.96	0.15	0.05	0.87	0.47
Kurds					
Alleles	4	8	16	6	18
MEC	0.37	0.58	0.77	0.51	0.81
PIC	0.59	0.75	0.87	0.69	0.90
HET±SE	0.66±0.03	0.79±0.03	0.88±0.02	0.74±0.03	0.91±0.02
PD(F)	0.81	0.92	0.97	0.89	0.98
p(HWE)	0.93	0.64	0.44	0.05	0.73

p(HWE) Exact test probability on Hardy–Weinberg expectations was performed by running 5,000 random shuffles, *MEC* mean exclusion chance, *PIC* polymorphism information content, *HET±SE* expected heterozygosity ± standard error, *PD(F)* power of discrimination for females, calculated according to http://www.chrx-str.org

1 µl of the DNA extract. Primer sequences, labeling, and references are depicted in "ESM Table S1". One microliter of PCR product was mixed with 12 µl formamide and 0.5 µl genRES LS500^{ROX} internal size standard (Serac, Bad Homburg, Germany).

Allele size determination was performed in an ABI PRISM 310 Genetic Analyzer (AB) by comparison with a sequenced allelic ladder (kindly provided by Peter M. Schneider, Cologne).

Sequencing of variant alleles and those affected by mutations was done as described previously [1]. To study the existence of isoalleles, some samples of the mutation cases were amplified and cloned into the pGEM® T Easy vector (Promega, Mannheim, Germany) according to the manufacturer's instructions.

For statistical analysis, the program HWE-Analysis (version 3.2, C. Puers, Münster) and online software (http://www.chrx-str.org) were used [13].

Results and discussion

Since the allelic distributions in men and in women were statistically not different, we pooled them for further analysis (ESM Table S2). All systems were in Hardy–Weinberg equilibrium (ESM Table S1). The genotype of cell line 9947A, which served as a positive control, was identical to the values published in [13] (data not shown).

Among the studied systems, DXS8377 was the most efficient and DXS8378 was the least informative (Table 1).

Table 2 Characteristics of the five mutation cases in the system DXS8377

Case no.	Child	Mother	Alleged father	Origin	Gain/loss
German 1	*49*	*51*/*51*	n.t.	Maternal	−2
German 2	*49*/*49*	49	*50*	Paternal	−1
Kurdish 1	*43*/49	49/50	50/*42*	Paternal	+1
Kurdish 2	47/*51*	47/51	*53*	Paternal	−2
Kurdish 3	44/*51*	*49*/54	44	Maternal	+2

Alleles affected by the mutational event are shown in italics.
n.t. Not available for typing

Table 3 Mutation rates and confidence intervals in both populations

		Paternal mutation rate (%)	95% C.I. (10^{-2})	Maternal mutation rate (%)	95% C.I. (10^{-2})
Germans	DXS6800	0	0.06–8.04	0	0.03–4.45
	DXS101	0	0.06–8.22	0	0.03–4.50
	DXS8377	2.3	0.56–12.02	1.3	0.30–6.69
Kurds	DXS6800	0	0.02–2.72	0	0.01–1.49
	DXS101	0	0.02–2.78	0	0.01–1.52
	DXS8377	1.5	0.47–5.33	0.4	0.10–2.24

C.I., according to http://www.causascientia.org/math_stat/ProportionCI.html

Table 4 Meiotic transfers in different age groups

Age	German population				Kurdish population			
	Paternal transfers	Mutations	Maternal transfers	Mutations	Paternal transfers	Mutations	Maternal transfers	Mutations
<14	0		3		0		0	
15–19	9		59	1	12		51	1
20–24	38		60		49		160	
25–29	24		21		78		210	
30–34	12	1	15		90	1	125	
35–39	0		3		38		45	
40–44	0		0		9		18	
45–49	0		0		15		3	
>50	0		0		3		0	
Unknown	0		0		42	1	46	
Total	83	1	161	1	336	2	658	1

The amplification of the locus DXS981 (STRX1) did not produce reproducible results for all the samples; thus, we decided not to report data for this system.

At the locus HPRTB, a variant allele, apparently 11.2, was detected in a German female. The sequence structure of this allele was determined as 12 (D48AGdel); hence, it should be called allele $12^{\#}$ according to the latest ISFG recommendations [14]. Similar allele sequences have already been described in an Italian [15] and a Portuguese [16] population, but never among Germans.

In the meioses study (Germans: 161 maternal and 83 paternal meiotic transmissions; Kurds: 658 maternal and 336 paternal meiotic transmissions) of the systems DXS6800, DXS101, and DXS8377, we observed a total of five mutations in the system DXS8377, two in the female and three in the male germ line. Among the Kurdish families, two two-step and one one-step mutations were identified, while in the German population, one two-step and one one-step mutations were identified. Repeat contractions were observed in three cases, repeat expansions in two cases (Table 2). Sequencing of the alleles affected by the mutational event revealed the possible presence of isoalleles. This assumption was confirmed for the two German mutation cases (Table 3). The apparent mutation rates (Table 3) can be regarded as initial estimates since the investigation of additional meiotic transfers is necessary. A correlation between the mutation rate and age of parents could not be observed (Table 4), but the number of mutations is yet too small to draw statistically significant conclusions.

When the forensic efficiency of the five systems was calculated, the combined power of discrimination among males [PD(M)] was no lower than 0.998, while among females the combined PD(F) was at least 0.9999 in both populations (Table 1). The combined power of paternity exclusion was a minimum of 0.998 in trio cases and 0.98 in motherless cases.

In conclusion, the five X-STRs systems have been shown to be highly informative in the two investigated populations.

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