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SHORT COMMUNICATION

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Population genetic comparisons of three X-chromosomal STRs

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Abstract The X-chromosomal short tandem repeats (STRs) DXS6800, DXS101 and DXS8377 were analysed in male and female population samples from Germany and Austria using a PCR multiplex approach. We investigated 135 family trios from Innsbruck (Austria) and surrounding areas and 50 families and further male and female samples from Ulm (Germany) and surrounding areas. The comparisons of the allele frequencies gave similar distributions for Innsbruck and Ulm although minor variations were found for some alleles. Additionally, some differences were found when comparing the allele frequencies of the male and female samples independently. The forensic efficiency values demonstrate that especially DXS101 and DXS8377 are highly informative markers for kinship analysis and deficiency cases. Based on the investigated meiotic events no new mutations were detected.

Keywords Short tandem repeats · X-chromosome · Population genetics · New mutations

Introduction

Autosomal STR polymorphisms for forensic analysis have been evaluated for many years [1, 2] and in addition, forensic interest has increasingly focused on Y-chromosomal STRs [3]. Meanwhile some studies on STRs located on the X-chromosome have been carried out which are of specific interest in paternity cases where the disputed child is female [4, 5, 6]. Such markers are especially important in deficiency cases because the mean exclusion chance of X-STRs tends to be higher than that of autosomal STR loci. If in a kinship case the question to be solved is whether two women who were separated as children could be sisters, exclusions can be detected using X-markers in contrast to autosomal STRs.

The aim of our study was to increase the pool of forensically relevant data for X-STR allele frequencies using a PCR multiplex approach including the three STR loci DXS6800 (4 bp repeat), DXS101 (3 bp repeat) and DXS8377 (3 bp repeat). In contrast to other published PCR protocols [5, 6, 7, 8] we generated a triplex PCR with modified primer sequences.

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Material and methods

The DNA samples were extracted using 5% Chelex 100 [9, 10] and from 200 µl extraction volume, 1–2 ng DNA was used for PCR.

The PCR protocol consisted of initial denaturation at 95°C for 11 min, 93°C for 60 s, 60°C for 60 s, 72°C for 60 s over 30 cycles using the published primer sequences for DXS101, DXS8377 [5, 8] and newly designed primers for DXS6800. Primers annealing more closely to the flanking region of the repeat array were selected which resulted in a 27 bp shorter amplicon length compared to the previously published primer sequences [8].

The primers for DXS6800 were:

– P1: 5' FAM-gtg gga cct tgt gat tgt gt
– P2: 5' aga acc tac gtt gaa ata ttg g

for DXS101 [5]:

– P1: 5' HEX-act cta aat cag tcc aaa tat ct
– P2: 5' aaa tca ctc cat ggc aca tgt at

and for DXS8377 [8]:

– P1: 5' FAM-cac ttc atg gct tac cac ag
– P2: 5' gac ctt tgg aaa gct agt gt

The PCR reaction components were: primer concentrations for DXS6800 5 pmol, for DXS101 8 pmol and for DXS8377 13 pmol each primer. PCR was carried out in a 12.5 µl reaction volume containing 1–2 ng DNA (Chelex extracted), 100 µM each dNTP, 5 µg BSA (Sigma 3350, München, Germany), 2.0 mM $MgCl_2$, 1.5 U

AmpliTaq-Gold, 1.25 µl AmpliTaqGold buffer II (ABI, Foster City, CA). The PCR products were analysed by capillary electrophoresis using ABI Prism 310 and 3100 sequencers (polymer POP 4). Allele typing was carried out based on sequenced alleles.

The following statistical analyses were carried out: polymorphism information content (PIC) [11], mean exclusion chance (MEC) [12], observed heterozygosity (HET), power of discrimination (PD) in females and males [13], Hardy-Weinberg equilibrium (HWE) and possible deviations from Hardy-Weinberg equilibrium were tested using an exact test (Genepop software http://wbiomed.curtin.edu.au/genepop).

Results and discussion

Individuals from two populations were investigated including 135 family trios from Innsbruck (Austria) and surrounding areas and 50 families and further female and male samples from Ulm (Germany) and surrounding areas. The allele frequencies of both population samples were calculated separately for females and males (Tables 1, 2, 3). Additionally, for each population pooled data for females and males were used to demonstrate the distributions of the alleles (Fig. 1a–c). The comparisons of the allele frequencies showed very similar distributions for Innsbruck and Ulm. In contrast, more obvious differences were found when comparing the allele frequencies of the male and female samples for some alleles (Table 1), e.g. DXS6800 allele 16. These deviations may be attributed to the smaller sample number of the male individuals.

For the statistical analysis and forensic efficiency data no deviations from Hardy-Weinberg equilibrium were found ($p > 0.05$), both population samples showed a similar very high, polymorphic information content (PIC) for DXS101 and DXS8377 (Tables 2, 3), and corresponding values for mean exclusion chance (MEC) and heterozy-

Table 1 DXS6800: allele frequencies and forensic efficiency values for male and female population samples from Germany and Austria

Allele	Germany		Austria	
	Female (*n*=216)	Male (*n*=108)	Female (*n*=270)	Male (*n*=134)
16	0.440	0.324	0.353	0.393
17	0.032	0.056	0.038	0.074
18	0.097	0.083	0.132	0.052
19	0.287	0.352	0.336	0.319
20	0.028	0.037	0.019	0.052
21	0.088	0.139	0.102	0.081
22	0.028	0.009	0.026	0.030
PIC	0.683	–	0.715	–
MEC	0.661	–	0.694	–
HET	0.648	–	0.735	–
PD	0.870	0.704	0.889	0.736

PIC polymorphism information content
MEC mean exclusion chance
HET observed heterozygosity
PD power of discrimination
n number of alleles

Table 2 DXS101: allele frequencies and forensic efficiency for male and female population samples from Germany and Austria values

Allele	Germany		Austria	
	Female (*n*=214)	Male (*n*=109)	Female (*n*=270)	Male (*n*=135)
15	0.033	0.018	0.023	0.015
16	0.005	0.000	0.004	0.000
17	0.005	0.009	0.000	0.000
18	0.079	0.064	0.090	0.059
19	0.047	0.073	0.026	0.037
20	0.019	0.028	0.015	0.030
21	0.033	0.028	0.011	0.030
22	0.023	0.018	0.015	0.007
23	0.056	0.064	0.060	0.037
24	0.229	0.211	0.237	0.274
25	0.164	0.128	0.169	0.200
26	0.140	0.174	0.154	0.141
27	0.089	0.073	0.086	0.074
28	0.033	0.064	0.064	0.081
29	0.023	0.037	0.034	0.015
30	0.023	0.000	0.008	0.000
31	0.000	0.009	0.000	0.000
PIC	0.871	–	0.857	–
MEC	0.865	–	0.850	–
HET	0.876	–	0.818	–
PD	0.974	0.877	0.968	0.864

Table 3 DXS8377: allele frequencies and forensic efficiency values for male and female population samples from Germany and Austria

Allele	Germany		Austria	
	female (*n*=220)	male (*n*=109)	female (*n*=270)	male (*n*=133)
37	0.005	0.000	0.008	0.007
38	0.014	0.000	0.000	0.000
39	0.018	0.018	0.023	0.037
40	0.018	0.046	0.034	0.044
41	0.064	0.036	0.041	0.044
42	0.045	0.083	0.049	0.058
43	0.036	0.101	0.053	0.058
44	0.032	0.064	0.083	0.029
45	0.095	0.055	0.080	0.080
46	0.118	0.138	0.105	0.088
47	0.182	0.055	0.120	0.110
48	0.114	0.110	0.102	0.131
49	0.095	0.110	0.086	0.102
50	0.073	0.073	0.080	0.066
51	0.045	0.055	0.061	0.059
52	0.018	0.028	0.041	0.051
53	0.018	0.018	0.026	0.022
54	0.000	0.009	0.004	0.015
55	0.009	0.000	0.004	0.000
56	0.000	0.000	0.004	0.000
PIC	0.900	–	0.920	–
MEC	0.897	–	0.918	–
HET	0.914	–	0.939	–
PD	0.983	0.904	0.989	0.923

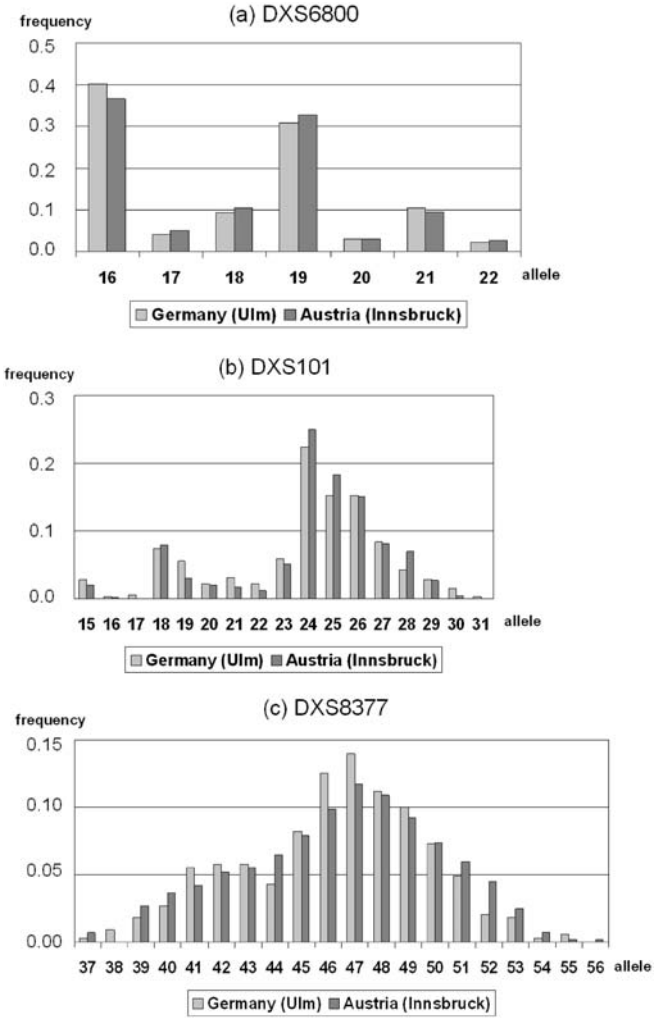

Fig. 1 Population genetic comparisons of allele frequency data for **a** DXS6800, **b** DXS101 and **c** DXS8377 from Germany and Austria based on pooled allele frequencies of female and male population samples (Table 1, 2, 3). The allele nomenclatures are aligned according to the number of repeats

Table 4 Distribution of the age of the parents at the time of conception [18]

Age at conception (years)	Numbers of mothers (%) (n=185)	Numbers of fathers (%) (n=93)
15–19	17.3	7.5
20–24	36.2	26.9
25–29	27.0	26.9
30–34	14.6	23.6
35–40	4.3	8.6
40–44	0.6	5.4
45–49	–	1.1

gosity (HET). For DXS101 a slightly lower heterozygosity was found for the Austrian samples. In contrast, a clearly lower heterozygosity value for DXS6800 was calculated in the German population, which corresponds to the high allele frequency of allele 16. In paternity analysis

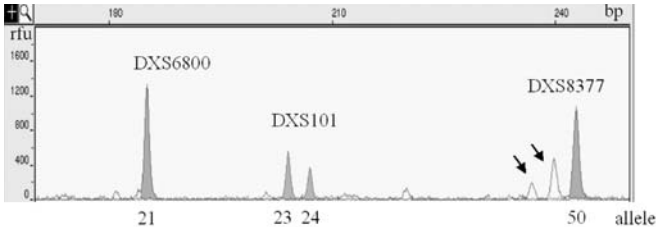

Fig. 2 Multiplex profile of the three X-STRs using 200 pg of K562 cell line DNA (Promega, Madison,WI) for PCR amplification over 30 cycles (*Rfu* relative fluorescence units). DXS8377 shows intensive stutter bands (*arrows*)

(see MEC values, Tables 2 and 3) DXS101 and DXS8377 were found to be more informative than commonly used autosomal STRs (e.g. HUMVWA, HUMFIBRA, D18S51 [14]).

Additionally, for paternity analysis and especially in deficiency cases it should be borne in mind that linkage has been verified between DXS101 and DXS6800 [15].

Based on the investigated meiotic events (185 maternal transfers, 93 paternal transfers (Table 4) no new mutations were detected. Although DXS101 and DXS8377 are characterised by relatively long repeat arrays (15–31 and 37–56, respectively), the mutation rate may be lower compared to other autosomal STRs containing long repeat stretches (e.g. HUMACTBP2 [16]), because the repeat arrays of both X-STRs show alternations of different repeat motifs [DXS101: $(CTT)_n$–$(ATT)_n$, DXS8377: $(GAA)_n$–$(GAG\text{-}GAA)_n$] [6, 8, 17]. Similarly, Edelmann and Szibor [5] reported no new mutations for DXS101 in 340 meioses.

Amplification of the K562 cell line DNA showed that for DXS6800 and DXS101 only minor stutter bands occurred. In contrast, for DXS8377 intensive stutter bands cannot be avoided because of the long repeat array (Fig. 2).

The three X-chromosome STRs are highly informative markers for kinship testing and especially DXS101 and DXS8377 are very useful for the investigation of deficiency cases.

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