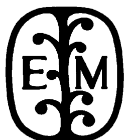

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Validation of the X chromosomal STR DXS7424 which is closely linked to DXS101

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Abstract

In some special cases of post-mortem identification and deficiency paternity testing, the investigation of X-chromosome (ChrX) markers may be more informative than the investigation of autosomal polymorphisms. We analysed the trinucleotide repeat polymorphism DXS7424 (GDB-G00-577-633), cytogenetically mapped at Xq22. PCR fragment length measurements, sequencing and linkage studies were carried out. In a sample of 474 unrelated Germans, we found 12 alleles with the regular repeat structure $(TAA)_{[9-20]}$. The population sample was characterised by the following features: polymorphism information content (PIC) = 0.776; heterozygosity (Het) = 0.836; mean exclusion chance (MEC) = 0.762. Kinship tests revealed a typical X-linked inheritance without any mutation. Significant deviations from the Hardy–Weinberg equilibrium (HWE) were not established. Linkage studies confirmed close linkage to DXS101. It was not possible to exclude linkage disequilibrium between DXS101 and DXS7424 through haplotyping of 390 male individuals.
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1. Introduction

In contrast to clinical genetics, the application of X-chromosome (ChrX) markers for forensic purposes plays only a marginal role to date. ChrX markers are only suitable for

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paternity testing when the disputed child is a girl. In cases of post-mortem identification and deficiency paternity testing where the alleged father cannot be typed, investigation of ChrX markers may yield the desired information. Autosomal and Y-chromosomal testing can be effectively supplemented by ChrX marker investigation. The trinucleotide repeat polymorphism DXS7424 is located in intron 5 of the human Bruton tyrosine kinase (BTK) gene [1] and cytogenetically mapped to Xq22.

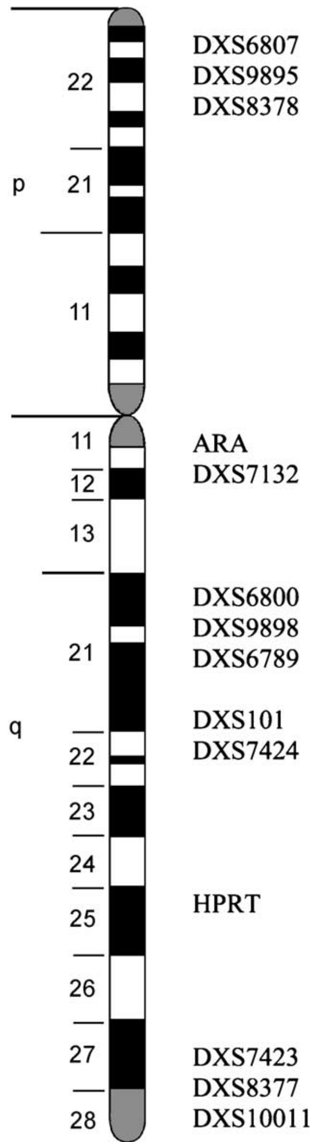

Fig. 1. ChrX idiogramm.

Application of genetic markers for forensic purposes requires knowledge of map position. Fig. 1 contains a ChrX ideogram depicting ChrX markers of forensic value. Validation results of several markers are shown: ARA [2–5], HPRTB [2,6,7], DXS6807 [8], DXS9898 [9], DXS6789 [10], DXS101 [11,12], and DXS10011 [13].

2. Materials and methods

DNA was extracted using QIAampDNABlood MiniKits (Qiagen, Hilden, Germany) from 474 unrelated Germans (220 females, 254 males) and 150 family trios with female children. The 25-μl PCR set-up contained 10-ng DNA in a mixture consisting of 1 U Taq (Perkin-Elmer, Foster City, CA), 0.25 μM each primer, 150 μM each nucleotide, 50 mM KCl, 10 mM Tris–HCl (pH 9.0) and 1.5 mM $MgCl_2$. PCR amplification was performed using primer sequences based on gene bank information (http://www.gdb.org).

2.1. Amplification protocol

95 °C—5 min, 28 cycles: 94 °C—30 s, 60 °C—30 s, 72 °C—75 s and 72 °C—6 min (final extension). The resulting PCR products were resolved and detected by capillary electrophoresis in the ABI 310 sequencer. Direct Taq-cycle-sequencing was carried out using the BigDye-Terminator Kit (Perkin-Elmer).

2.2. Statistical analysis

Hardy–Weinberg equilibrium (HWE) was tested following the exact testing method [14]. Various parameters were calculated: polymorphism information content (PIC) [15], heterozygosity (Het) [16], mean exclusion chance (MEC) [4] and average power of discrimination (PD^F, PD^M) in females and in males [3].

2.3. Linkage analysis

Radiation hybrid (RH) mapping was performed using the Stanford G3-RH-Panel RH01 (Research Genetics, Huntsville, AL, USA). The results were checked by means of the Stanford Human Genome RH Server (http://shgc.stanford.edu).

Several ChrX markers were genetically localised by using integrated maps of other gene banks (http://www.gdb.org, http://www.chlc.org, http://www.ncbi.nlm.nih.gov). The NCBI map contains data from the International RH Mapping Consortium [17] and indicates genetic localisation of markers as cM distances from the top of the chromosomes.

3. Results and discussion

Allele frequencies were calculated separately for males and females (Table 1 and Fig. 2). A number of 29 different genotypes were found in females (data not shown). Sequencing of randomly selected alleles, including the adjacent single-copy sequences,

Table 1
Allele frequencies for DXS7424

Allele	Length (bp)	n	Females ($n=220$) proportion	n	Males ($n=254$) proportion	n	Pooled alleles proportion
9	147	1	0.00227 ± 0.0023	0	0	1	0.00144 ± 0.0014
10	150	2	0.00454 ± 0.0032	1	0.00399 ± 0.0039	3	0.00432 ± 0.0025
11	153	1	0.00227 ± 0.0023	3	0.01181 ± 0.0067	4	0.00576 ± 0.0029
12	156	17	0.03863 ± 0.0092	10	0.03937 ± 0.0122	27	0.03890 ± 0.0073
13	159	38	0.08636 ± 0.0134	12	0.04724 ± 0.0133	50	0.07204 ± 0.0098
14	162	112	0.25454 ± 0.0208	40	0.15748 ± 0.0228	152	0.21902 ± 0.0156
15	165	131	0.29773 ± 0.0218	79	0.31102 ± 0.0290	210	0.30259 ± 0.0174
16	168	86	0.19545 ± 0.0189	69	0.27165 ± 0.0279	155	0.22334 ± 0.0158
17	171	38	0.08636 ± 0.0134	30	0.11811 ± 0.0202	68	0.09798 ± 0.0113
18	174	7	0.01591 ± 0.0060	7	0.02756 ± 0.0103	14	0.02017 ± 0.0053
19	177	7	0.01591 ± 0.0060	2	0.00787 ± 0.0055	9	0.01297 ± 0.0043
20	180	0	0	1	0.00399 ± 0.0039	1	0.00144 ± 0.0014

PIC: 0.764 MEC: 0.762
$Het_{obs.}$: 0.836 $Het_{exp.}$: 0.793
PD^F: 0.927 PD^M: 0.791

PIC: polymorphism information content [15]; MEC: mean exclusion chance [4]; Het: heterozygosity [16]; PD^F: average power of discrimination in females [3]; PD^M: PD in males [3].

revealed a regular sequence composition with the repeat structure $(TAA)_{[9-20]}$. Alleles were assigned in compliance with the recommendations of the ISFH Commission [18]. Twelve alleles were identified, increasing in size by 3 bp increments in a range from 147 to 180 bp and designated from 9 to 20. The K562 control DNA displayed the allele 17.

Significant deviations from the Hardy–Weinberg equilibrium were not found: the exact test yielded a p-value of 0.335.

The parameters of interest (PIC, Het, MEC, PD^F and PD^M) shown in Table 1 confirm that DXS7424 is a highly informative marker. Investigations in 150 family trios with female children suggested X-linked inheritance. No mutations were found.

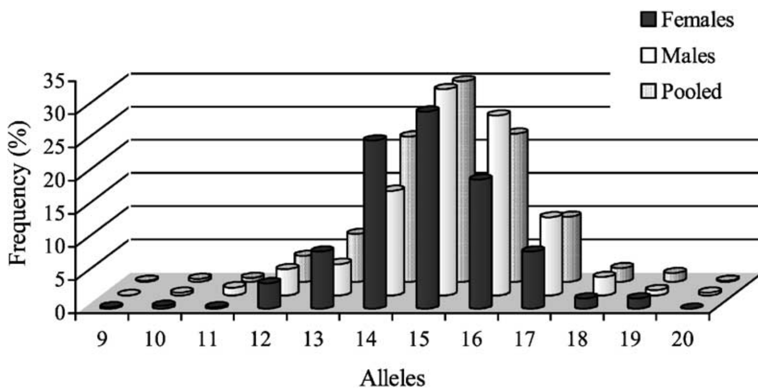

Fig. 2. Allele frequencies for DXS7424.

The RH-mapping procedure for DXS7424 yielded eight closely linked SHGC markers, which were mapped to DXS7424 within the given lod-score of 4. They were mapped into the anchor marker interval DXS990 to DXS1059. This corresponds to a genetical localisation of 104.9–121 cM and a cytogenetical position in Xq22. DXS101 mapping yielded identical results [12]. Hence, DXS7424 and DXS101 are very closely linked. Allele frequency data of DXS101 have been published recently [12]. To test for association, we performed a Monte Carlo two-sided *p*-value with its 99% confidence interval in 390 ChrX haplotypes of males. The results indicated linkage disequilibrium between DXS101 and DXS7424 ($p = 0.0063$ (0.0043,0.0083); StatXact-4). Therefore, it is inadmissible to calculate haplotype frequencies using their single allele frequencies. Haplotype frequencies have to be estimated directly by population studies. Table 2 shows 67 DXS7424–DXS101 haplotypes and their upper frequencies found in 390 males. In kinship testing, this haplotype frequency (mean frequency plus standard deviation) should be used to guarantee conservative calculations of relationship probability.

Summarising our data, we conclude that DXS7424–DXS101 is a highly informative ChrX-marker complex qualified for ChrX haplotyping in complicated kinship tests. The pronounced linkage disequilibrium between these markers is not only in accordance with very tight linkage but also confirms our observation of a very low mutation rate in both markers.

Table 2
DX7424–DXS101 haplotypes of 390 males

Haplotype 7424-101	Upper frequency	Haplotype 7424-101	Upper frequency	Haplotype 7424-101	Upper frequency
10–15	0.005125	14–28	0.033645	16–24	0.045318
10–25	0.005125	14–29	0.005125	16–25	0.065276
11–24	0.008745	14–30	0.005125	16–26	0.042423
11–29	0.005125	15–15	0.018517	16–27	0.018517
12–24	0.005125	15–18	0.036588	16–28	0.024672
12–25	0.008745	15–19	0.033645	16–29	0.008745
12–26	0.030680	15–20	0.005125	17–15	0.005125
12–28	0.012116	15–22	0.008745	17–16	0.008745
13–17	0.005125	15–23	0.015358	17–18	0.021617
13–24	0.015358	15–24	0.082085	17–19	0.005125
13–25	0.027690	15–25	0.036588	17–23	0.015358
13–26	0.024671	15–26	0.095948	17–24	0.039514
13–27	0.008745	15–27	0.024672	17–25	0.033645
13–28	0.005125	15–28	0.030680	17–26	0.005125
14–18	0.024671	15–30	0.012116	17–30	0.005125
14–19	0.012116	16–15	0.008745	18–18	0.021617
14–21	0.005125	16–16	0.008745	18–19	0.005125
14–22	0.005125	16–18	0.024671	18–24	0.018517
14–23	0.021617	16–19	0.042423	18–29	0.005125
14–24	0.053929	16–20	0.015358	19–15	0.008745
14–25	0.027690	16–21	0.018517	20–23	0.005125
14–26	0.018517	16–22	0.012116		
14–27	0.039514	16–23	0.005125		

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