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SHORT COMMUNICATION

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DXS101: a highly polymorphic X-linked STR

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Abstract This paper presents sequence and population genetic data for the microsatellite marker DXS101 which is a highly polymorphic X-linked trinucleotide polymorphism with 18 alleles 179–233 bp in length. A polymorphism information content (PIC) of 0.884 and a mean exclusion chance (MEC) of 0.879 were obtained by analysing a Caucasian population sample. A deviation from the Hardy-Weinberg equilibrium (HWE) could not be detected. Kinship tests revealed a typical X-linked inheritance and no mutations were found in 340 meioses. DXS101 is located 104.9–121 cM from the Xp-telomere (Xp-tel) corresponding to Xq21.33–Xq22.3. Concomitant testing of DXS101 and DXS6807 is possible as these two markers are unlinked. The data presented qualify this X-linked microsatellite marker as a useful tool for forensic purposes.

Keywords Short tandem repeat · X-chromosome · Kinship testing

Introduction

A large number of autosomal DNA polymorphisms have been evaluated for forensic use and widely applied to stain analysis and kinship testing (Urquhart et al. 1994; Brinkmann 1998). In addition, forensic interest has increasingly focused on Y-chromosomal markers over the past few years (Jobling et al. 1997; Kayser et al. 1997). However, only a small number of X chromosome (ChrX) markers have been available, and in paternity testing, ChrX markers are only applicable when the disputed child is female. In such cases an X-chromosomal marker has a higher mean exclusion chance (MEC, Krüger et al. 1968) than an autosomal marker with a comparable PIC value. Kishida et al. (1997) modified Krüger et al.'s original formula for ChrX marker application which leads to a higher MEC value due to the fact that males are diploid for all autosomes but hemizygous for ChrX markers.

Only four X-linked STR markers have been comprehensively described for forensic applications, the trinucleotide polymorphism ARA (Kishida and Tamaki 1997; Desmarais et al. 1998), and the tetranucleotide STRs HPRTB (Kishida et al. 1997; Mertens et al. 1999; Szibor et al. 2000), DXS9898 (Hering and Szibor 2000) and DXS6807 (Edelmann and Szibor 1999). This paper aims at adding the new marker DXS101 chosen because of the high degree of polymorphism resulting from 18 alleles.

DXS101 is a human sequence-tagged site (STS) first published by Allen and Belmont (1993).

Materials and methods

DNA was extracted from the blood of 564 unrelated Germans (348 females, 216 males) using the QIAamp DNA blood kit (Qiagen, Hilden, Germany), and 170 family trios including female children were checked for regular X-chromosomal inheritance (340 meioses). The parental age structure was as follows: 96 mothers and 53 fathers aged between 15 and 25 years, 68 mothers and 92 fathers aged between 25 and 35 years, and 6 mothers and 18 fathers aged between 35 and 45 years; no mothers and 7 fathers were older than 45 years.

PCR amplification was performed using the following primer sequences based on gene bank information (http://www.gdb.org):

- Primer I: 5′-(6′-FAM)-ACT CTA AAT CAG TCC AAA TAT CT-3′
- Primer II: 5′-AAA TCA CTC CAT GGC ACA TGT AT-3′

Amplifications were carried out in a 25 µl PCR reaction volume containing 10 ng DNA, 200 µM each dNTP, 1.5 mM $MgCl_2$, 0.5 µM each primer, 1 U Taq (Perkin-Elmer, Foster City, Calif.) and 1 × PCR buffer for 30 cycles using a thermocycler (Biometra, Göttingen, Germany) and 95 °C with a 10 -min soak, 94 °C for 60 s, 58 °C for 60 s, 72 °C for 90 s and 72 °C for a 10 min final extension.

The resulting PCR products were resolved and detected by capillary electrophoresis in the denaturing polymer POP 4 (Perkin-

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Elmer, Foster City, Calif.) in the ABI Prism 310 sequencer following standard protocols. Fragment sizing was supported using the GeneScan 500 TAMRA size standard.

Sequencing analysis

Male DNA samples were employed to produce PCR products for use as sequencing templates. Sequencing reactions were performed after removing excess primers and nucleotides from the PCR reaction mixture, using the method of polyethylene glycol (PEG) precipitation (Rosenthal et al. 1993). Direct Taq-cycle-sequencing was carried out using the BigDye-Terminator kit (Perkin-Elmer, Foster City, Calif.) and the unlabelled PCR primer I at a concentration of 3 pmol. Sequence data were obtained from 27 random alleles.

Linkage analysis

Radiation hybrid (RH) mapping was performed using the Stanford G3-RH-Panel RH01 (Research Genetics, Huntsville, Ala.). The presence or absence of a specific PCR product in each radiation hybrid was determined in triplicate by performing the PCR procedure for DXS101. The results were checked by means of the Stanford Human Genome RH Server (http://shgc.stanford.edu) to obtain information about the DXS101 linkage to the SHGC marker backbone.

Genetic localisation of this and further ChrX markers which could possibly be used concomitantly were established by using integrated maps of other gene banks (http://www.gdb.org, http://www.chlc.org, http://carbon.wi.mit.edu, http://www.ncbi.nlm.nih.gov). The NCBI map contains data from the International RH Mapping Consortium (Deloukas et al. 1998) and indicates genetic localisation of markers as cM distances from the top of the chromosomes.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was examined using the exact test (Guo and Thompson 1992). The polymorphism information content (PIC) and heterozygosity (H) were calculated as suggested by Botstein et al. (1980) and Nei and Roychoudhury (1974), respectively. The mean exclusion chance in normal family trio tests (MEC) was computed as proposed by Kishida et al. (1997), the paternity exclusion chance (PE), the average power of discrimination in females (PD^F) and in males (PD^M) were calculated according to Desmarais et al. (1998).

Results and discussion

Table 1 shows the DXS101 allele frequencies of a German population sample calculated separately for males and females whereby 18 alleles were identified with length variation which increased in size by 3 bp-increments ranging from 179–233 bp. Sequencing of 27 randomly selected alleles revealed a regular sequence composition (Table 2). Alleles were assigned in compliance with the recommendations of the ISFH Commission (Bär et al. 1997). The proposed designation of DXS101 alleles ranged from 14 for a 179 bp-allele to 32 for a 233 bp-allele. We developed an allelic ladder for genotyping consisting of nine alleles. The control DNA K562 which can be employed for calibrating, displayed the allele 24.

The polymorphic region is formed by two blocks consisting of variable numbers of trinucleotide repeat motifs, i.e. CTT and ATT. Alleles which are identical in size re-

Table 1 DXS101 – Allele frequencies and standard errors in 348 females and 216 males (n_{pooled} 564). *PIC* Polymorphism information content 0.884 (Botstein et al. 1980), *MEC* mean exclusion chance 0.879 (Kishida et al. 1997), *PE* expected probability of exclusion 0.794 (Desmarais et al. 1998), *H* heterozygosity H_{exp} 0.890, H_{obs} 0.885 ± 0.017 (Nei and Roychoudhury 1974), PD^F average power of discrimination in females 0.978 (Desmarais et al. 1998), PD^M PD in males 0.889 (Desmarais et al. 1998)

Allele	Size (bp)	Males		Females		Homozygotes	Pooled alleles	
		n	Frequency	*n*	Frequency		*n*	Frequency
14	179	–	0.000 ± 0.000	2	0.003 ± 0.002	–	2	0.002 ± 0.001
15	182	8	0.037 ± 0.013	32	0.046 ± 0.008	–	40	0.044 ± 0.007
16	185	1	0.005 ± 0.005	4	0.006 ± 0.003	–	5	0.005 ± 0.002
17	188	–	0.000 ± 0.000	2	0.003 ± 0.002	–	2	0.002 ± 0.001
18	191	17	0.078 ± 0.018	60	0.086 ± 0.011	2	77	0.084 ± 0.009
19	194	15	0.069 ± 0.017	28	0.040 ± 0.007	1	43	0.047 ± 0.007
20	197	3	0.014 ± 0.008	8	0.011 ± 0.004	–	11	0.012 ± 0.004
21	200	9	0.042 ± 0.014	20	0.029 ± 0.006	–	29	0.032 ± 0.006
22	203	3	0.014 ± 0.008	17	0.024 ± 0.006	1	20	0.022 ± 0.005
23	206	10	0.046 ± 0.014	50	0.072 ± 0.010	3	60	0.066 ± 0.008
24	209	47	0.217 ± 0.028	146	0.210 ± 0.015	16	193	0.212 ± 0.013
25	212	30	0.139 ± 0.023	112	0.161 ± 0.014	8	142	0.156 ± 0.012
26	215	32	0.148 ± 0.024	72	0.103 ± 0.011	3	104	0.114 ± 0.011
27	218	19	0.088 ± 0.019	53	0.076 ± 0.010	4	72	0.079 ± 0.009
28	221	16	0.074 ± 0.018	48	0.069 ± 0.010	2	64	0.070 ± 0.008
29	224	2	0.009 ± 0.006	23	0.033 ± 0.007	–	25	0.027 ± 0.005
30	227	4	0.018 ± 0.009	18	0.026 ± 0.006	–	22	0.024 ± 0.005
32	233	–	0.000 ± 0.000	1	0.001 ± 0.001	–	1	0.001 ± 0.001
Total		216		696		40	912	

Table 2 Allele nomenclature, sequence composition and fragment length of 27 sequenced PCR fragments (*Pr.1* sequence of primer 1, *Pr.2* complementary sequence of primer 2, *N* flanking region nucleotides)

Allele	Fragment length	Sequence composition	Alleles sequenced
15	182 bp	Pr.1-N_{38}-$(CTT)_6$-$(ATT)_9$-N_{53}-Pr.2	1
16	185 bp	Pr.1-N_{38}-$(CTT)_6$-$(ATT)_{10}$-N_{53}-Pr.2	1
18	191 bp	Pr.1-N_{38}-$(CTT)_8$-$(ATT)_{10}$-N_{53}-Pr.2	1
	191 bp	Pr.1-N_{38}-$(CTT)_7$-$(ATT)_{11}$-N_{53}-Pr.2	1
21	200 bp	Pr.1-N_{38}-$(CTT)_{17}$-$(ATT)_4$-N_{53}-Pr.2	1
22	203 bp	Pr.1-N_{38}-$(CTT)_{16}$-$(ATT)_6$-N_{53}-Pr.2	1
23	206 bp	Pr.1-N_{38}-$(CTT)_{15}$-$(ATT)_7$-N_{53}-Pr.2	1
24	209 bp	Pr.1-N_{38}-$(CTT)_{15}$-$(ATT)_9$-N_{53}-Pr.2	1
	209 bp	Pr.1-N_{38}-$(CTT)_{12}$-$(ATT)_{12}$-N_{53}-Pr.2	2
	209 bp	Pr.1-N_{38}-$(CTT)_{14}$-$(ATT)_{10}$-N_{53}-Pr.2	1
	209 bp	Pr.1-N_{38}-$(CTT)_{17}$-$(ATT)_7$-N_{53}-Pr.2	2
	209 bp	Pr.1-N_{38}-$(CTT)_{19}$-$(ATT)_5$-N_{53}-Pr.2	1
25	212 bp	Pr.1-N_{38}-$(CTT)_{13}$-$(ATT)_{12}$-N_{53}-Pr.2	2
	212 bp	Pr.1-N_{38}-$(CTT)_{16}$-$(ATT)_9$-N_{53}-Pr.2	2
	212 bp	Pr.1-N_{38}-$(CTT)_{19}$-$(ATT)_6$-N_{53}–Pr.2	2
	212 bp	Pr.1-N_{38}-$(CTT)_{18}$-$(ATT)_7$-N_{53}-Pr.2	1
26	215 bp	Pr.1-N_{38}-$(CTT)_{14}$-$(ATT)_{12}$-N_{53}-Pr.2	2
	215 bp	Pr.1-N_{38}-$(CTT)_{15}$-$(ATT)_{11}$-N_{53}-Pr.2	1
	215 bp	Pr.1-N_{38}-$(CTT)_{17}$-$(ATT)_9$-N_{53}-Pr.2	1
28	221 bp	Pr.1-N_{38}-$(CTT)_{17}$-$(ATT)_{11}$-N_{53}-Pr.2	1
30	227 bp	Pr.1-N_{38}-$(CTT)_{19}$-$(ATT)_{11}$-N_{53}-Pr.2	1

vealed considerable structural variations with regard to the CTT/ATT content. Experience with well-established STRs, such as ACTBP2 (Möller and Brinkmann 1994) suggests that sequence variability does not disturb the accuracy of fragment length measurement when denaturing electrophoretic techniques are used. Thus, sequence heterogeneity in STRs does not cause any disadvantage, but establishes additional hidden information which can be utilised by sequencing in very special cases. In 348 females, 83 genotypes were counted (data not shown)and the distribution did not indicate any deviation from the Hardy-Weinberg equilibrium (p = 0.651). The HWE check was performed by the exact test by binning of alleles (data not shown). Kinship tests in 170 family trios with female children suggested regular X-linked codominant inheritance.

Although a strong correlation between the repeat number and mutation rate has been reported in the literature (Wierdl et al. 1997), mutations have not as yet been observed in DXS101. A high microsatellite stability is attributed to an interrupted repeat structure and irregular interspersed repeats (Petes et al. 1997; Brinkmann et al. 1998). The CTT/ATT structure divides the DXS101 STR into two parts.

The RH-mapping procedure for DXS101 yielded six SHGC markers, i.e. SHGC-7399, SHGC-57323, SHGC-15676, SHGC-37409, SHGC-16117, and SHGC-1237 mapped to DXS101 within the given lod score of 4 and indicated a physical distance of 0–11 cR. The International RH Mapping Consortium mapped three of them into the anchor marker interval from DXS990 to DXS1059 which corresponds to a genetic localisation of 104.9–121 cM from Xp-tel and a cytogenetic position in Xq22.

Within the same mapping system ARA is mapped into the interval of DXS991–DXS1194 corresponding to 86.9–87.6 cM. HPRTB is localised into the anchor marker bin DXS1047–DXS998 corresponding to 150.3–183.8 cM from the ChrX top. DXS6807 is not included in the NCBI map, however the Whitehead ChrX map localised it to 13.2 cM from the Xp-tel.

Molecular localisation of the DXS9898 locus is described with DXS991–DXS990. This corresponds to a ChrX bin size of 86.9–104.9 cM from Xp-tel. These details suggest that the genetic distance between DXS101 and ARA and HPRTB is 18–34 cM and 29.3–78.9 cM, respectively. Hence, there is a rather loose linkage between DXS101 and ARA which, however, is not certain between DXS101 and HPRTB. In addition, there is a verified linkage between DXS101 and DXS9898. The DXS6807 locus is at least 91 cM away from DXS101, i.e. these markers are unlinked and suitable for concomitant use in kinship testing without any limitations.

Comprehensive investigations will be required before DXS101 can be used concomitantly with other X-linked markers.

The reported data suggest that DXS101 is a highly informative marker which should be preferably employed for kinship testing. Its length variation is caused by regular trinucleotide increments. As a result of an X-linked inheritance, DXS101 yields an MEC of the same order of magnitude as that of one of the most informative autosomal markers, i.e. ACTBP2. In addition to parentage testing of female children, ChrX markers are very useful in cases of deficiency paternity.

References

Allen RC, Belmont JW (1993) Trinucleotide repeat polymorphism at DXS101. Hum Mol Genet 2: 1508

Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA Commission of the ISFH DNA recommendations – Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 110: 175–176

Botstein D, White RI, Skolnich M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314–331

Brinkmann B (1998) Overview of PCR-based systems in identity testing. Methods Mol Biol l98: 105–119

Brinkmann B, Klintschar M, Neuhuber F, Hühne J, Rolf B (1998) Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. Am J Hum Genet 62: 1408–1415

Deloukas P, Schuler GD, Gyapay G, Beasley EM, Soderlung C, Podriguez-Tomé P, et al. (1998) Physical map of 30,000 human genes. Science 282: 744–746

Desmarais D, Zhong Y, Chakraborty R, Perreault C, Busque L (1998) Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). J Forensic Sci 43: 1046–1049

Edelmann J, Szibor R (1999) Validation of the HumDXS6807 short tandem repeat polymorphism for forensic application. Electrophoresis 20: 2844–2846

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361–372

Hering S, Szibor R (2000) Development of the X-linked tetrameric microsatellite marker DXS9898 for forensic purposes. J Forensic Sci 45: 929–931

Jobling MA, Pandya A, Tyler-Smith C (1997) The Y chromosome in forensic analysis and paternity testing. Int J Legal Med 110: 118–124

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

Kishida T, Tamaki Y (1997) Japanese population data on X-chromosomal STR locus AR. Nippon Hoigaku Zasshi 51: 376–379

Kishida T, Wang W, Fukuda M, Tamaki Y (1997) Duplex PCR of the Y-27H39 and HPRT loci with reference to Japanese population data on the HPRT locus. Nippon Hoigaku Zasshi 51: 67–69

Krüger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung der sauren Erythrozytenphosphatase bei der Vaterschaftsbegutachtung. Dtsch Z Gerichtl Med 64: 127–146

Mertens G, Gielis M, Mommers N, Mularoni A, Lamartine J, Heylen H, Muylle L, Vandenberghe A (1999) Mutation of repeat number of the HPRTB locus and structure of rare intermediate alleles. Int J Legal Med 112: 192–194

Möller A, Brinkmann B (1994) Locus ACTBP2 (SE33) Sequencing data reveal considerable polymorphism. Int J Legal Med 106: 262–267

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. Genetics 76: 379–390

Petes TD, Greenwell PW, Dominska M (1997) Stabilization of microsatellite sequences by variant repeats in the yeast *Saccharomyces cerevisiae*. Genetics 146: 491–498

Rosenthal A, Coutelle O, Craxton M (1993) Large-scale production of DNA sequencing templates by microtitre format PCR. Nucleic Acids Res 21: 173–174

Szibor R, Lautsch S, Plate I, Beck N (2000) Population data of X chromosomal STR HumHPRTB in two regions of Germany. J Forensic Sci 45: 231–233

Urquhart A, Kimpton CP, Downes TJ, Gill P (1994) Variation in short tandem repeat sequences – a survey of twelve microsatellite loci for use as forensic identification markers. Int J Legal Med 107: 13–20

Wierdl M, Dominska M, Petes TC (1997) Microsatellite instability in yeast: dependence on the length of microsatellite. Genetics 146: 769–779

ANNOUNCEMENT

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