BRIEF COMMUNICATION

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Development of the X-Linked Tetrameric Microsatellite Marker DXS9898 for Forensic Purposes

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ABSTRACT: HumDXS9898 also known as CHLC·GATA 126G01 is a tetrameric microsatellite marker located at the Xq21.33 pericentromeric region. In kinship testing HumDXS9898 is suitable for concomitant use with HumHPRTB and HumDXS6807 which are separated from HumDXS9898 by genetic map distance of 150 and 80 cM, respectively. HumDXS9898 is closely linked to HumARA. In the German population, HumDXS9898 exhibits seven clearly distinguishable alleles ranging from 189 to 214 basepairs in size. Deviation from Hardy-Weinberg equilibrium could not be detected. The observed heterozygosity was 0.75 for females and the mean exclusion probability was 0.73 for female children. Mutations were not found in the present material.

KEYWORDS: forensic science, paternity testing, short tandem repeat, X chromosome, HumDXS9898 (GATA126G01), Germany

Autosomal STR markers are widely applied to forensic stain analysis, postmortem identification, and paternity testing. However, in some special cases of postmortem identification through kinship testing and deficiency paternity testing, the investigation of non-autosomal markers may be more informative than the investigation of autosomal polymorphisms. Deficiency paternity testing means that the alleged father cannot be typed and the examiner investigates the relatives of the alleged father. The advantage of non-autosomal testing is that males transmit an X chromosome to all of their daughters and a Y chromosome to all of their sons. To our knowledge, only three X-linked markers have been described for forensic applications, HumHPRTB (located at Xq26.1) (1–7), HumARA (located at Xcen–q13) (1,2,8–11), and Hum DXS6807 (located at Xpter—p22.2) (12). The aim of this paper is to add the STR HumDXS9898 to the panel of forensically used X markers.

HumDXS9898 (also known as CHLC·GATA126G01) is an unpublished human sequence-tagged site (dbSTS:16780) submitted to the Cooperative Human Linkage Centre in 1995 by Murray J, Sheffield V, Weber JL, Duyk G, Buetow KH. It is located on the X chromosome at DXS991—DXS990. This is equal to an X chromosome bin size of 86.9 to 104.9 cM and a cytogenetical localization of Xq21.33. HumARA is located between DXS991 and DXS983, corresponding to 86.9 to 94.4 cM. Thus, HumDXS9898 and HumARA are closely linked markers and are not suited for concomitant use but should be used alternatively. HumHPRTB and HumDXS6807, which are located at a distance of about 150 and 80 cM from DXS9898 respectively, can be used for typing and subsequent statistical analysis. (All information for localization indicated above were taken from the NCBI gene bank.)

Allele designation was done after sequencing 33 HumDXS9898 PCR fragments. The repeat structure and allele distribution were studied in an East German population sample.

Materials and Methods

Blood for DNA extraction was obtained from 492 unrelated Germans, i.e., 245 females and 247 males. One hundred thirty-nine family trios including female children were checked for regular X-chromosomal inheritance.

The 25 μL PCR set-up contained 0.2–1 ng DNA in a buffer consisting of 1 U AmpliTaqDNApolymerase and 1x Buffer II (Perkin Elmer, Foster City, CA), 0.5 μM each primer, 200 μM each nucleotide (Pharmacia, Upsala, Sweden), 50 mM KCl, 10 mM Tris-HCl (pH 9.0) and 2.5 mM $MgCl_2$. Primers were synthesized by Applied Biosystems GmbH, Weiterstadt, Germany.

Primer 1,5′-FAM (5-carboxy-fluorescein) labeled: 5′-CGAG-CACACCTACAAAAGCT-3′ Primer 2: 5′-TCGATTAG-GTTCAGTTCCCA-3′. Amplification was carried out in a PTC-200 cycler (MJ Research Inc, Watertown, MA). The cycle conditions were: 95°C-3 min soak, 94°C-30 s, 62°C-1 min, 72°C-1 min, 30 cycles, 72°C-10 min final extension. The resulting PCR products were resolved and detected by capillary electrophoresis in the denaturing polymers POP4 (Perkin-Elmer, Foster City, CA) in the ABI 310 sequencer (Perkin-Elmer, Foster City, CA) following standard protocols. Fragment sizing was supported using the Genescan™ 500 TAMRA size standard (Perkin-Elmer, Foster City, CA).

Sequencing Analysis

Both strands of hemizygote PCR products were sequenced with the direct Taq-cycle-sequencing method using the BigDye-Termi-

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nator Kit (Perkin Elmer, Foster City, CA) and the unlabeled PCR primers 1 and 2, respectively. When rare alleles were not available in the hemizygote state, PCR products were separated from heterozygote females using native horizontal polyacrylamide gel electrophoresis (T 6.4%, C 3.3%; gel thickness 500 μm, gel length 25 cm; 600 V, 5–8 W, 3–4 h). The Multiphor II electrophoresis equipment (Pharmacia, Upsala, Sweden) was employed. Bands of interest were dissected, eluted and reamplified. To remove excessive primers and nucleotides from the PCR reaction prior to sequencing, we used CENTRI-SEP columns (Princeton Separations, Adelphia, NJ).

Twelve μL final volume of sequencing mixture contained 2.5 μL reaction mix, 1.5 μL primer (1 pmol/μL) and 40–80 ng PCR product. Twenty-five cycles were carried out as follows: 95°C - 5 s, 60°C - 90 s, 50°C - 90 s. Cycle sequencing products were separated in a 47 cm capillary under module Seq POP6 Rapid (1 mL) E, 25 min run time, 30 s injection time. Data were analyzed by means of the PE/ABD software Sequencing Analysis 3.0 (Perkin Elmer, Foster City, CA).

Statistical analyses of the population data to detect any deviation from the Hardy-Weinberg equilibrium (HWE) were performed by an exact test (13) using the DNAVIEW software package version 21.06 (Brenner C. 1998, Berkeley, CA).

The observed heterozygosity was compared with the unbiased estimate of the expected heterozygosity (14). The suitability of the DXS9898 locus for paternity analysis (testing of a girl) and identity testing was assessed by calculating the mean exclusion chance for X-linked codominant inheritance (9) and the power of discrimination (15). Afterwards, the PIC was calculated (16).

Results and Discussion

The allele distribution of the German population sample used for this study is shown in Table 1. Seven different alleles are exhibited. As the HumDXS9898 locus revealed an X-linked codominant inheritance, we calculated the frequencies for males and females separately.

The repeat composition of the seven alleles is displayed in Table 2. Fragment lengths ranged from 189 bp to 214 bp. The alleles were designated by the number of the tetranucleotide repeats according to the International Society of Forensic Haemogenetics (ISFH) guidelines (17). One ATC-motif obviously conserved within the repeat array (in italics) was not included in the allele nomenclature. The smallest allele exhibited a second incomplete repeat, i.e., ATC-trinucleotide which was named allele 8.3. The K562 control DNA exhibits the HumDXS9898 allele 12 and can be used for calibrating allelic ladders.

The observed genotypes and the results of the analyses of the population data with regard to their accordance with the HWE-hypothesis are shown in Table 3. The differences between the observed and expected phenotype frequencies in females gives a value of $p = 0.082$ (with first standard deviation confidence interval) after the application of an exact test (5000 random shuffles performed). The value of the observed heterozygosity was within the range defined by the expected value (±1.96 standard error). Hence, we concluded that there was no evidence of significant deviations from the genetic equilibrium.

We investigated 239 mother-child and 162 father-daughter meiosis. No mother-child exclusions were found, nor were exclusions detected in those cases where paternity had previously been confirmed. The mean exclusion chance for female children was 0.73. The average discrimination powers at this locus were 0.91 and 0.72 for testing samples of female and male origin, respectively. The PIC was calculated as 0.73.

The reported population genetic data of the DXS9898 polymorphism suggest that this marker is of interest for forensic analyses. Further population genetic studies should be conducted with the aim to confirm a low mutation rate, which would qualify this

TABLE 1—*HumDXS9898 allele frequencies from 247 unrelated males and 245 females.*

Allele Designation	Male Number	Male Proportion	Female Number	Female Proportion	Cumulated Frequency
8.3	66	0.267 ± 0.028	132	0.269 ± 0.020	0.269 ± 0.016
10	2	0.008 ± 0.006	4	0.008 ± 0.004	0.008 ± 0.003
11	54	0.219 ± 0.026	108	0.220 ± 0.019	0.220 ± 0.015
12	75	0.304 ± 0.029	140	0.286 ± 0.020	0.292 ± 0.017
13	38	0.154 ± 0.023	77	0.157 ± 0.016	0.156 ± 0.013
14	12	0.049 ± 0.014	25	0.051 ± 0.010	0.050 ± 0.008
15	0	0	4	0.008 ± 0.004	0.005 ± 0.003

TABLE 2—*Allele nomenclature, fragment length and repeat composition of 33 sequenced PCR fragments (alleles 8.3–15). This table gives the fragment lengths as can be counted by means of the sequencing data.*

Allele Designation	Fragment Length (bp)	Sequence Structure			Number of Sequenced Alleles
8.3	189	$(TATC)_2$ (*ATC*)	(TATC) (ATC)	$(TATC)_5$	5
10	194	$(TATC)_2$ (*ATC*)		$(TATC)_8$	4
11	198	$(TATC)_2$ (*ATC*)		$(TATC)_9$	5
12	202	$(TATC)_2$ (*ATC*)		$(TATC)_{10}$	7
13	206	$(TATC)_2$ (*ATC*)		$(TATC)_{11}$	4
14	210	$(TATC)_2$ (*ATC*)		$(TATC)_{12}$	5
15	214	$(TATC)_2$ (*ATC*)		$(TATC)_{13}$	3

TABLE 3—*HumDXS9898 genotypes found in 217 females.*

Allele	8.3	10	11	12	13	14	15
8.3	20						
10	3	0					
11	26	0	15				
12	31	0	36	22			
13	28	0	13	18	5		
14	4	1	3	8	7	1	
15	0	0	0	3	1	0	0

HWE exact test: $p = 0.0818$ (0.07792–0.08568).
Observed heterozygosity: 0.74.
Expected heterozygosity: 0.772 (0.719–0.824).

marker as an useful tool for solving complicated cases of kinship testing.

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