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SHORT COMMUNICATION

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Meiosis study in a population sample from Afghanistan: allele frequencies and mutation rates of 16 STR loci

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Abstract The 16 short tandem repeat systems D3S1358, VWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, ACTBP2, D2S1338, D16S539, D19S433, D21S11, D18S51 and D8S1179 were amplified in a population sample composed of 333 immigrants from Afghanistan. The 16 loci met Hardy–Weinberg expectations and possess a combined matching probability of 1 in 3.6×10^{14} and a combined mean exclusion chance greater than 0.9996 in this Afghan population. Approximately 12,000 meiotic transfers were investigated and 19 mutations were observed in the repeat units of FGA (n=6), ACTBP2 (n=5), D3S1358 (n=2), D5S818 (n=2), D7S820 (n=2), VWA (n=1) and D8S1179 (n=1).

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

Introduction

The aim of this work was to establish a database for the Afghan population for forensic purposes including paternity testing. We present here the allele frequencies, mutation rates and forensic efficiency values for the 16 STR loci D3S1358, VWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, ACTBP2, D2S1338, D16S539, D19S433, D21S11, D18S51, and D8S1179 in a sample of 333 unrelated Afghan immigrants, 169 men and 164 women, seeking asylum in Germany.

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Materials and methods

Genomic DNA was extracted from oral cotton swab samples by the proteinase K/Chelex method [1]. The 16 different STR systems were amplified using various kits (e.g. AmpF*l*STR Profiler and Identifiler [2] PCR amplification kits (Applied Biosystems, Darmstadt, Germany), Power ES (Promega, Mannheim, Germany), MPX3-SE ([3] Serac, Bad Homburg, Germany). Typing was performed using denaturing capillary gel electrophoresis on an ABI PRISM 310 Genetic Analyzer according to the manufacturer's instructions.

Evaluation of Hardy–Weinberg expectations and other forensic statistical parameters was done with the computer programme HWE-Analysis 3.2 (Chr. Puers, Münster). Observed de novo mutations were included in the biostatistical evaluation according to Essen–Möller and, if necessary, additional STR systems were typed to reach a paternity probability value $W \geq 99.997\%$.

Variant alleles and alleles from the mutation cases were isolated as described elsewhere [4] and directly sequenced using BigDye Terminator Cycle Sequencing Kit (ABI) with primers for both strands to check if the mutation had occurred in the repeat array.

Results and discussion

No deviation from Hardy–Weinberg equilibrium was observed for the 16 STR loci (Table 1). These 16 systems show a combined matching probability of 1 in 3.6×10^{14} and a combined mean exclusion chance greater than 0.9996 in the Afghan population investigated. According to these statistical parameters, this combination is a powerful tool for forensic identification and paternity testing.

Table 1 Characteristics of the 19 mutation cases from Afghanistan

Case no.	System	Child	Mother	Alleged father	Origin	Gain/loss	Sequence
E068/2004	ACTBP2	**16**/**29.2**	n.t.	**17**/**28.2**	Paternal	+1/−1	$(AAAG)_{10->11}$ AAAAAG $(AAAG)_{17}$ or $(AAAG)_{17->16}$
E042/2002a	ACTBP2	20/**31.2**	20/25.2	30.2/**32.2**	Paternal	−1	$(AAAG)_{13->12}$ AAAAAG $(AAAG)_{18}$
E260/2003	ACTBP2	20/**31.2**	17/20	20.2/**32.2**	Paternal	−1	$(AAAG)_{12}$ AAAAAG $(AAAG)_{19->18}$
E065/2004	ACTBP2	14/**26.2**	14/27	**25.2**	Paternal	+1	$(AAAG)_{12->13}$ AAAAAG $(AAAG)_{12}$ or $(AAAG)_{13}$ AAAAAG $(AAAG)_{11->12}$
E097/2001	ACTBP2	16/**19**	n.t.	**18**/26.2	Paternal	+1	$(AAAG)_{18->19}$
E147/2001	D5S818	**13**	11/13	**12**	Paternal	+1	$(GATA)_{12->13}$ GAT
E174/2004	D5S818	9/**12**	9/14	**11**/**13**	Paternal	+1/−1	$(TATC)_{11->12}$ or $(TATC)_{13->12}$
E247/1998	D3S1358	18/**19**	**18**	16/**18**	Paternal/maternal	+1	$(AGAT)_{13->14}$ $(AGAC)_3$ $(AGAT)_2$
E246/2001	D3S1358	**17**	15/**18**	17/18	Maternal	−1	$(AGAT)_{13->12}$ $(AGAC)_3$ $(AGAT)_2$
E271/1998	D7S820	**11**/12	8/**12**	9/**12**	Paternal/maternal	−1	$(GATA)_{12->11}$
E422/2002	D7S820	11/**12**	8/**11**	8/**11**	Paternal/maternal	+1	$(GATA)_{11->12}$
E069/2002	VWA	17/**19**	17	15/**18**	Paternal	+1	TCTA $(TCTG)_4$ $(TCTA)_{13->14}$
E112/2003	D8S1179	**14**	13/14	**13**/**15**	Paternal	+1/−1	$(TATC)_{15->14}$ or $(TATC)_{13->14}$
E062/2002	FGA	**23**/24	**24**/25	**24**/26	Paternal/maternal	−1	$(TTTC)_3$ TTTT TTCT $(CTTT)_{16->15}$ CTCC $(TTCC)_2$
E233/2001	FGA	**22**/25	20/**23**	19/25	Maternal	−1	$(TTTC)_3$ TTTT TTCT $(CTTT)_{15->14}$ CTCC $(TTCC)_2$
E296/2001	FGA	**23**/25	n.t.	21/**22**	Paternal	+1	$(TTTC)_3$ TTTT TTCT $(CTTT)_{14->14}$ CTCC $(TTCC)_2$
E002/2003	FGA	**23**/24	**24**/25	**24**	Paternal/maternal	−1	$(TTTC)_3$ TTTT TTCT $(CTTT)_{16->15}$ CTCC $(TTCC)_2$
E346/2002	FGA	21/**24**	21/26	21/**25**	Paternal	−1	$(TTTC)_3$ TTTT TTCT $(CTTT)_{17->16}$ CTCC $(TTCC)_2$
E259/2004	FGA	20/**23**	20/22	21/**24**	Paternal	−1	$(TTTC)_3$ TTTT TTCT $(CTTT)_{16->15}$ CTCC $(TTCC)_2$

n.t. Not available for typing
alleles affected by the mutational event are shown in bold

Table 2 Mutation rates of the 16 STR systems in Afghanistan

System	Paternal mutations	Maternal mutations	Unassigned	Paternal transmission	Maternal transmissions	Paternal mutation rate [%]	95% CI [%]	Maternal mutation rate [%]	95%CI [%]
D3S1358	0	1	1	482	456	0.00	0.01–0.76	0.22	0.05–1.21
VWA	1	0	0	480	456	0.21	0.05–1.15	0.00	0.01–0.80
FGA	3	1	2	482	456	0.62	0.23–1.80	0.22	0.05–1.21
TH01	0	0	0	482	456	0.00	0.01–0.76	0.00	0.01–0.80
TPOX	0	0	0	482	456	0.00	0.01–0.76	0.00	0.01–0.80
CSF1PO	0	0	0	482	456	0.00	0.01–0.76	0.00	0.01–0.80
D5S818	2	0	0	482	456	0.41	0.13–1.49	0.00	0.01–0.80
D13S317	0	0	0	482	456	0.00	0.01–0.76	0.00	0.01–0.80
D7S820	0	0	2	482	456	0.00	0.01–0.76	0.00	0.01–0.80
D16S539	0	0	0	179	165	0.00	0.01–2.03	0.00	0.02–2.20
D2S1338	0	0	0	179	165	0.00	0.01–2.03	0.00	0.02–2.20
D8S1179	1	0	0	299	273	0.33	0.08–1.84	0.00	0.01–1.34
D21S11	0	0	0	299	273	0.00	0.01–1.22	0.00	0.01–1.34
D18S51	0	0	0	300	273	0.00	0.01–1.22	0.00	0.01–1.34
D19S433	0	0	0	179	165	0.00	0.01–2.03	0.00	0.02–2.20
ACTBP2	5	0	0	385	356	1.30	0.57–3.00	0.00	0.01–1.03
Total	12	2	5	6156	5774	0.19	0.11–0.34	0.035	0.01 –0.13

Table 3 Age distribution in mutation cases from Afghanistan

Age [years]	Paternal mutations	Paternal cases	Maternal mutations	Maternal cases
<19	0	13	0	38
20–24	0	55	0	128
25–29	3	115	1	142
30–34	5	141	0	97
35–39	3	82	1	43
>40	1	78	0	11
Total	12	484	2	459

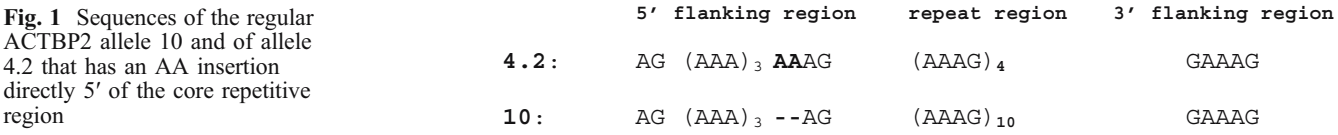

Fig. 1 Sequences of the regular ACTBP2 allele 10 and of allele 4.2 that has an AA insertion directly 5′ of the core repetitive region

A total of 19 one-step mutations were observed under approximately 12,000 meiotic transfers, 12 in the male and 2 in the female germ line, while five mutations could not be assigned. The ratio of repeat gains and losses was relatively balanced (7:9), while three mutations could not be assigned (Table 2). The observed mutation rates were in the range from 0 to 1.3×10^{-2} per locus per gamete per generation (Table 3) and, thus, are in the range reported by Brinkmann et al. [5] for Germans.

A slight increase of the mutation rate with age could be observed (Table 3), but the numbers are too low and do not allow statistically significant conclusions to be drawn.

In the hypervariable system ACTBP2 allele 4.2, the second smallest allele known at present and included in commercially available ladders (e.g. [6]) was observed nine times. Sequencing revealed a AA insertion directly 5′ of the core repetitive region of ACTBP2 (Fig. 1). As this allele 4.2 has not yet been found in other populations investigated (e.g. [1, 7]), it may help to determine the population of origin if detected in a stain from an unknown donor.

Sequencing also helped to assign the mutational events, e.g. in case E042/2002a, the filial allele 31.2 might have originated from the paternal alleles 30.2 or 32.2. Sequencing of allele 31.2 revealed the following motif $(AAAG)_{12}$ AAAAAG $(AAAG)_{18}$, which is compatible with the paternal allele 32.2 [motif $(AAAG)_{12}$ AAAAAG $(AAAG)_{19}$] but incompatible with the allele 30.2 [motif $(AAAG)_{10}$ AAAAAG $(AAAG)_{20}$ + a deletion of AAAG in the 5′ flanking region]. Thus, this mutation could be classified as a paternal one-step loss.

In case E112/2003, the presence of iso allele 14 at D8S1179 in the child [$(TATC)_2$ TGTC $(TATC)_{11}$ and $(TATC)_{14}$] assisted in assigning the mutation to the paternal germ line because the mother inherited the iso allele 14 [$(TATC)_2$ TGTC $(TATC)_{11}$], while the father's repeat structures were $(TATC)_{13}$ and $(TATC)_{15}$. This could be either a repeat gain or loss but they cannot be distinguished.

To conclude, we have established a forensic database for allele frequencies and mutation rates in the Afghan population that is useful for identity and parentage testing.

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