International Congress Series 1261 (2004) 236–238

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Short tandem repeat polymorphisms across the HLA-complex: sequence and population data of D6S389 and D6S1051

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Abstract. The tetranucleotide repeat loci D6S389 and D6S1051 situated nearby the HLA class II region (6p) were investigated in an Austrian Caucasoid population sample. Typing of the amplification products and cycle sequencing were carried out using denaturing capillary electrophoresis. For D6S389 and D6S1051, 19 and 7 different alleles were observed. Sequencing of D6S389 revealed a $(GAAA)_n$ repeat pattern in the common alleles. Additionally, infrequent ".1" alleles were seen. Alleles of D6S1051 showed a simple $(GATA)_n$ repeat structure and an A/G-SNP next to the repeat region as well as a T/C-SNP in the 3′ -flanking region. D6S389 proved to be a highly polymorphic marker. D6S1051 is less polymorphic, but interesting because of the existing SNPs. © 2003 Published by Elsevier B.V.

Keywords: D6S389; D6S1051; SNP

1. Introduction

The tetranucleotide repeat loci D6S389 (GenBank L16321, [1]) and D6S1051 (GenBank G08553) situated nearby the HLA class II region (6p) were investigated in an Austrian Caucasoid population sample ($n = 153$) in order to get information about their usefulness in identity and paternity testing or as HLA haplotype markers.

2. Materials and methods

DNA was extracted from peripheral blood lymphocytes (DNA Blood Mini Kit, Qiagen). PCR was carried out in a reaction volume of 15 μl using 0.4 μM of each primer,

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0531-5131/ © 2003 Published by Elsevier B.V.
doi:10.1016/S0531-5131(03)01719-9

Table 1
D6S389 and D6S1051-allele frequencies and statistic parameters ($n = 153$)

Allele designation	Allele frequencies		Number of sequenced alleles	
	D6S389	D6S1051	D6S389	D6S1051
8	0.056		6	
9	0.026	0.003	6	1
10	0.042	0.065	7	9
11	0.082	0.248	6	10
12	0.163	0.386	6	11
13	0.141	0.243	9	12
13.1	0.010		4	
14	0.150	0.052	12	10
14.1	0.003		1	
15	0.121	0.003	11	1
16	0.098		9	
16.1	0.010		1	
17	0.042		7	
18	0.029		5	
18.1	0.003		1	
19	0.010		4	
21	0.007		1	
22	0.003		1	
24	0.003		1	
Rate of heterozygosity	0.915	0.647		
Power of exclusion	0.826	0.351		
Polymorphism information content	0.880	0.680		
Power of discrimination	0.972	0.883		
Typical paternity index	5.880	1.420		

Table 2
Sequence structure of STR locus D6S389

Allele						Length (bp)
8–24	CAAGAAA	$[(GAAA)\ GGAAA]_3$	$(GAAA)_{8-22;24}$	GAA	GAAAAA	287–351
13.1	CAAGAAA	$[(GAAA)\ GGAAA]_4$	$(GAAA)_{11}$	GAA	GAAAAA	308
14.1	CAAGAAA	$[(GAAA)\ GGAAA]_3$	$(GAAA)_{14}$	GAAA	GAAAAA	312
15.1	CAAGAAA	$[(GAAA)\ GGAAA]_4$	$(GAAA)_{13}$	GAA	GAAAAA	316
16.1	CAAGAAA	$[(GAAA)\ GGAAA]_3$	$(GAAA)_{16}$	GAAA	GAAAAA	320
18.1	CAAGAAA	$[(GAAA)\ GGAAA]_3$	$(GAAA)_{18}$	GAAA	GAAAAA	328

Table 3
Sequence structure of STR locus D6S1051

Allele							Length (bp)
9–13	$(GATA)_2$ TAGA	**C**ATA	GATAGAT	$(GATA)_{9-13}$	... AAAAAAA	T**T**AT	218–234
11–15	$(GATA)_2$ TAGA	**C**GTA	GATAGAT	$(GATA)_{11-15}$	... AAAAAAA	T**C**AT	226–242
13	$(GATA)_2$ TAGA	**T**GTA	GATAGAT	$(GATA)_{13}$	... AAAAAAA	T**C**AT	234

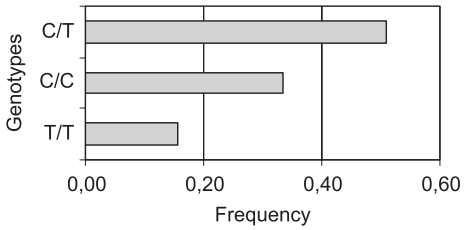

Fig. 1. Distribution of T/C-SNP genotypes in the 3′ flanking region of STR D6S1051.

200 μM dNTPs, 1 × Buffer II, 1.5 mM $MgCl_2$ and 0.5 units AmpliTaq Gold® DNA polymerase for 11 min at 95 °C, 28 cycles of 20 s 94 °C, 1 min 61 °C, 1 min 72 °C and a final extension of 45 min 60 °C (9700 thermal cycler).

Typing of the amplification products in comparison with locus-specific sequenced allelic ladders was carried out using denaturing capillary electrophoresis on an ABI Prism 310® Genetic Analyzer.

SNP analysis in the 3′ flanking region of D6S1051 (ss6492059) employing an "Assay-by-Design" (Applied Biosystems) was performed according to the manufacturer's instructions on an ABI 7700 Sequence Detection System.

3. Results and discussion

Typing for D6S389 and D6S1051 revealed 19 and 7 different alleles. The allele frequencies observed as well as further statistic data are shown in Table 1. No deviation from Hardy–Weinberg equilibrium has been detected.

Sequencing of D6S389 alleles from the current study population and another series of samples showed a $(GAAA)_n$ in the common alleles. Additionally, infrequent ".1" alleles were observed. The different sequence structures are shown in Table 2.

Alleles of D6S1051 showed a simple $(GATA)_n$ repeat structure and an A/G-SNP next to the repeat region as well as a T/C-SNP in the 3′ flanking region. In addition, one sample had a C>T transition on the 66th position of the 5′ flanking region (Table 3).

Analysis of the SNP in the 3′ flanking region of D6S1051 with alleles T and C revealed frequencies of 0.4 and 0.6, respectively. The distribution of genotypes is shown in Fig. 1.

4. Conclusion

D6S389 turned out to be a highly polymorphic marker, which can be used for identity analysis as well as for paternity testing. D6S1051 is less polymorphic, but nevertheless interesting because of the existing SNPs.

Reference

[1] A. Foissac, et al., Microsatellite markers in the HLA region, Tissue Antigens 55 (6) (2000) 477–509.