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The tetranucleotide repeat polymorphism C 2_4_4: sequence and population data

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Abstract

The STR locus C 2_4_4 situated in the HLA class I region (6p21.3) was investigated in an Austrian Causasoid population sample of 247 unrelated individuals. Beside length polymorphism, the alleles at this locus also revealed sequence polymorphism.

Keywords: C2_4_4; HLA class I region; Population study; Sequencing

1. Introduction

The tetranucleotide repeat STR locus C2_4_4 is situated in the HLA class I region (6p21.3) near the HLA-B and HLA-C locus. Polymorphic microsatellite markers within the HLA region have various application potentialities and are therefore of great interest. Our investigation concentrated on Austrian population and sequence data at this locus.

2. Materials and methods

A total of 247 unrelated Austrian Caucasoid individuals were included in the study. The salting out procedure was applied to isolate DNA from blood. The primer sequences were chosen according to Tamiya et al. [1] and PCR reactions were carried out in a

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volume of 50 μl with 4–8 ng template DNA, 2 U Amplitaq Gold DNA polymerase (Applied Biosystems), 0.8 μM of each primer (reverse primer Fluorescein labelled), 200 μM of each nucleotide and 1 × PCR buffer (GeneAmp®10 × PCR Buffer, Applied Biosystems) overlaid with 50 μl of paraffin oil. Amplification conditions were chosen as follows: 93 °C 9 min for 1 cycle; 94 °C 1 min, 54.3 °C 1 min, 72 °C 1 min 30 s for 34 cycles; 72 °C 10 min for 1 cycle (Thermocycler: Hybaid omnigene). Denaturing polyacrylamide gel electrophoresis with automated detection by laser induced fluorescence was carried out on an A.L.F. DNA sequencer according to the manufacturer's instructions in a 0.5-mm gel using the former commercially available polyacrylamide gel solution Acryl-a-Mix®6 (Promega) and 0.6 × TBE as running buffer. Sequencing and the composition of an allelic ladder used for typing was performed as described by Glock et al. [2].

3. Results and discussion

In our population sample, a total of 13 different alleles ranging in size from 227 to 275 bp could be differentiated by electrophoresis. Frequencies of the discovered alleles and further important statistical data are shown in Table 1. In chi-square analysis, no deviations from Hardy–Weinberg expectations could be detected ($p = 0.906$). Beside length polymorphism, the alleles at this locus also revealed sequence polymorphism (Table 2). The GenBank sequence (Accession No. AC004196) corresponds to allele 17 with a GAAA-GAAG-$(GAAA)_{11}$-AAAA-$(GAAA)_3$ repeat pattern. The calculated stat-

Table 1
SE33: allele frequencies and further statistical data obtained by denaturing electrophoresis ($n = 135$)

Allele designation	Allele frequency	Allele designation	Allele frequency
12	0.007	22.2	0.019
12.2	0.004	23.2	0.033
13	0.007	24.2	0.044
13.2	0.004	25.2	0.052
14	0.026	26.2	0.037
15	0.037	27.2	0.093
16	0.052	28.2	0.041
17	0.074	29.2	0.067
18	0.070	30.2	0.088
19	0.070	31.2	0.037
20	0.052	32.2	0.015
20.2	0.011	34	0.004
21	0.022	34.2	0.007
21.2	0.015	35.2	0.004
22	0.004	36.2	0.004
Rate of heterozygosity: 0.932		Matching probability: 0.013	
Power of exclusion: 0.861		Power of discrimination: 0.987	
Polymorphism information content: 0.95		Typical paternity index: 8.44	

Table 2
C2_4_4: sequence variations

Allele designation	Length in bp	Repeat structure	Position 79 3′ FR	Number of sequenced alleles
9	227	$(GAAA)_6$-AAAA-$(GAAA)_2$	A	7
		$(GAAA)_5$-AAAA-$(GAAA)_3$	A	1
10	231	$(GAAA)_6$-AAAA-$(GAAA)_3$	A	6
11	235	$(GAAA)_7$-AAAA-$(GAAA)_3$	A	5
13	243	GAAA-GAAG-$(GAAA)_7$-AAAA-$(GAAA)_3$	G	5
14	247	GAAA-GAAG-$(GAAA)_8$-AAAA-$(GAAA)_3$	G	1
15	251	GAAA-GAAG-$(GAAA)_9$-AAAA-$(GAAA)_3$	G	3
16 (−3)	252[a]	GAAA-GAAG-$(GAAA)_{10}$-AAAA-$(GAAA)_3$	G	1
16	255	GAAA-GAAG-$(GAAA)_{10}$-AAAA-$(GAAA)_3$	G	5
		GAAA-GAAG-$(GAAA)_{14}$	G	1
17	259	GAAA-GAAG-$(GAAA)_{11}$-AAAA-$(GAAA)_3$	G	1
		GAAA-GAAG-$(GAAA)_{12}$-AAAA-$(GAAA)_2$	G	2
		GAAA-GAAG-$(GAAA)_6$-GGAA-$(GAAA)_4$-AAAA-$(GAAA)_3$	G	3
18	263	GAAA-GAAG-$(GAAA)_{12}$-AAAA-$(GAAA)_3$	G	5
		GAAA-GAAG-$(GAAA)_{13}$-AAAA-$(GAAA)_2$	G	1
19	267	GAAA-GAAG-$(GAAA)_{13}$-AAAA-$(GAAA)_3$	G	3
20	271	GAAA-GAAG-$(GAAA)_{14}$-AAAA-$(GAAA)_3$	G	4
21	275	GAAA-GAAG-$(GAAA)_{15}$-AAAA-$(GAAA)_3$	G	3

[a] Deletion of 3 bp at positions 19–21 in the 5′ flanking region.

istical parameters show that this marker is approximately as powerful as HumTH01 [3] and is therefore suitable for application in paternity testing and human identification.

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