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Multiplex PCR investigation of the STR loci C1_4_4, C2_4_4 and C3_3_6 in the HLA class I region

S. Wenda[a,*], E.M. Dauber[a], S. Stadlbacher[a], B. Glock[b], G. Dorner[a], W.R. Mayr[a]

[a] *Department of Blood Group Serology and Transfusion Medicine, Division of Blood Group Serology, University of Vienna, Waehringer Guertel 18-20, Vienna, 1160 Austria*

[b] *Laboratory for Molecular Biology, Austrian Red Cross, Blood Donation Center for Vienna, Lower Austria and Burgenland, Vienna, Austria*

Abstract. In this study, we investigated the short tandem repeat loci C1_4_4, C2_4_4 and C3_3_6 situated in the HLA class I region in a sample of 153 unrelated Austrian Caucasoid individuals by multiplex PCR. The sequence structure of C1_4_4 and C3_3_6 alleles is described, as well as the sequence of a new C2_4_4 allele, allele 23.

Keywords: C1_4_4; C2_4_4; C3_3_6; HLA class I region; Multiplex PCR

1. Introduction

Sequence and population data of the short tandem repeat loci C1_4_4, C2_4_4 and C3_3_6, situated in the HLA class I region (6p21.3) near the HLA-B and HLA-C locus, were investigated in this study in order to find out, if they are suitable for identity and paternity testing.

2. Materials and methods

DNA was extracted from peripheral blood lymphocytes (Qiagen) of 153 unrelated Austrian Caucasoid individuals. The forward primers were labelled with fluorescein and the reverse primers were modified to so-called ''PIGtail'' primers to achieve complete 3′ A addition [1,2]. PCR conditions were chosen as follows (reaction volume 10 μl): 1–2 ng template DNA, 0.8 μM (C1_4_4), 0.2 μM (C2_4_4) and 0.4 μM (C3_3_6) of each primer, 200 μM of each nucleotide, 0.33 U Amplitaq Gold DNA polymerase, 1 × GeneAmp PCR Buffer; 95 °C 11 min for 1 cycle; 94 °C 1 min, 60 °C 1 min, 72 °C 2 min for 30 cycles; 72 °C for 45 min and 4 °C hold (GeneAmp 9700 PCR System, Applied Biosystems).

* Corresponding author. Klinische Abteilung für Blutgruppenserologie, A-1090 Wien, Waehringer Guertel 18-20, Austria. Tel.: +43-1-40400-5320; fax: +43-1-40400-5321.

E-mail address: sabine.wenda@univie.ac.at (S. Wenda).

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Table 1
Allele frequencies and further statistic parameters

Allele	C1_4_4	C2_4_4	C3_3_6
7	0.215		
8	0.131		0.003
9	0.042	0.095	0.395
10	0.065	0.490	0.183
11	0.046	0.020	0.036
12	0.036		0.206
13	0.059	0.013	0.056
14	0.059	0.003	0.020
15	0.023	0.003	0.072
16	0.056	0.150	0.026
17	0.065	0.092	0.003
18	0.056	0.075	
19	0.072	0.036	
20	0.069	0.013	
21	0.003	0.007	
22	0.003		
23		0.003	
Rate of heterozygosity	0.856	0.699	0.771
Power of exclusion	0.707	0.427	0.547
Polymorphism information content	0.890	0.690	0.730
Matching probability	0.023	0.109	0.097
Power of discrimination	0.977	0.891	0.903
Typical paternity index	3.480	1.660	2.190

Table 2
C1_4_4 sequence structure

Allele	5′ Flanking region position						Repeat structure	Length in bp	Number of sequenced alleles
	50	94	109	226	227	260–262			
7	G	C	G	G	T	AAA	$(GAAA)_7$	380	1
8	G	C	G	G	C	AAA	$(GAAA)_8$	384	2
9	G	A	G	A	C	AAA	$(GAAA)_9$	388	1
10	G	C	G	G	C	AAA	$(GAAA)_{10}$	392	1
11	G	A	G	A	C	AAA	$(GAAA)_{11}$	396	1
12	G	A	A	G	C	AAA	$(GAAA)_{12}$	400	2
13	G	A	A	G	C	AAA	$(GAAA)_{13}$	404	1
14	G	A	A	G	C	AAA	$(GAAA)_{14}$	408	1
15	G	A	G	A	C	AAA	$(GAAA)_{15}$	412	1
16	G	A	G	A	C	AAA	$(GAAA)_{16}$	416	1
16′	A	A	G	A	C	AAA	$(GAAA)_{16}$	416	1
17	A	A	G	A	C	AAA	$(GAAA)_{17}$	420	1
18	A	A	G	A	C	AAA	$(GAAA)_{18}$	424	1
19	G	A	G	A	C	AAA	$(GAAA)_{19}$	428	1
20	G	A	G	A	C	AAA	$(GAAA)_{20}$	432	1
21	G	A	G	A	C	AAA	$(GAAA)_{20}$ $(TAAA)_1$	436	1
22	G	A	G	A	C	AAA	$(GAAA)_{22}$	440	1

Table 3
C3_3_6 sequence structure

Alleles	5′ Flanking region pos. 53	Repeat structure	3′ Flanking region pos. 47	pos. 89	Length in bp	Number of sequenced alleles
8	G	$(TTA)_8$	T	A	287	1
9–10	G	$(TTA)_{9-10}$	T	T	290–293	2
11	G	$(TTA)_{11}$	C	A	296	1
12	G	$(TTA)_{12}$	T	T	299	1
13–14	G	$(TTA)_{13-14}$	C	A	302–305	2
15–16	A	$(TTA)_{15-16}$	C	A	308–311	2
17	G	$(TTA)_{17}$	C	A	314	1

Amplification products were analyzed on an ABI Prism 310 Genetic Analyzer. The alleles were assigned by comparison with a sequenced allelic ladder. A template was worked out with the *Genotyper* software to assign allele designations automatically. Cycle sequencing, on an ABI Prism 310 Genetic Analyzer, was performed.

3. Results and discussion

In our population sample 16 alleles at the C1_4_4 locus (380–440 bp), 13 alleles at the C2_4_4 locus (227–283 bp) and 10 alleles at the C3_3_6 locus (287–314 bp) could be differentiated. Allele frequencies and further statistic parameters are shown in Table 1. No deviation from Hardy-Weinberg equilibrium has been found.

A tetranucleotide repeat structure $(GAAA)_{7-22}$ was observed at the C1_4_4 locus (Table 2). Additionally, five SNPs were found. All alleles sequenced differed from the GenBank sequence (Accession No. AC006048) due to 3 instead of 4 A following the position 259 in the 5′ flanking region. Sequence data of the C2_4_4 locus $(RRAR)_n$ have already been described elsewhere [3,4]. Additionally, a new C2_4_4 allele, allele 23, exhibiting the sequence structure GAAA GAAG $(GAAA)_{17}$ AAAA $(GAAA)_3$ was detected.

At the C3_3_6 locus, a trinucleotide repeat structure $(TTA)_{8-17}$ was observed. The allele 9 sequenced in this study was identical with the GenBank sequence (Accession No. AC004207). Among the other alleles, three polymorphic sites (SNPs) were found (Table 3).

C1_4_4 has been found to be a powerful marker, which can be compared with FGA. C2_4_4 and C3_3_6 are approximately as informative as THO1.

These loci are therefore suitable for identity and paternity testing. The linkage disequilibrium between these alleles and HLA class I alleles is under study, as well as the possibility to use C1_4_4, C2_4_4 and C3_3_6 as HLA haplotype markers.

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