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BRIEF COMMUNICATION

Twenty-three novel HLA-B alleles identified during intermediate-resolution testing

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Abstract

Twenty-three novel human leukocyte antigen-B alleles are described: B*070204, *0738, *0742, *0821, *130202, *1312, *1575, *1598, *1599, *270507, *2728, *350104, *3558, *3811, *3931, *3932, *4045, *4107, *420501, *4812, *510106, *5520, and *5616. Thirteen of the variants are single-nucleotide substitutions from their most homologous allele, eight resulting in amino acid changes (B*0742, *1312, *1598, *1599, *3558, *3931, *4107, and *5616) and five with silent substitutions (B*070204, *130202, *270507, *350104, and *510106). Three alleles (B*0738, *4812, and *5520) differ by five nucleotide changes, altering four amino acids. The remaining seven alleles differ from their most similar alleles by two to three nucleotides, altering from one to two amino acids.

Twenty-three novel human leukocyte antigen (HLA)-B locus alleles were detected as unexpected hybridization patterns during intermediate-resolution DNA-based typing. The majority of individuals were initially typed at intermediate resolution either by a sequence-specific oligonucleotide probe bead-based technology (One Lambda LABType®) SSO Kit (One Lambda, Canoga Park, CA, USA) or using probes designed in-house (1). Two samples were typed low resolution by sequence specific primers. The cells carrying novel alleles are listed in Table 1.

Table 2 compares the novel sequences with the sequences of the most homologous alleles. Several unique features of specific novel alleles should be noted. B*270507 introduces a new site of polymorphism, codon 161. Six new polymorphisms were found altering codons already described as polymorphic for the HLA-B locus. Three were silent mutations (B*070204, *130202, and *510106) and three resulted in new amino acid substitutions (B*0742, *1312, and *1575). For example, codon 57 is polymorphic in HLA-B alleles, being found as three alternative codons [CCG (Pro), CGG (Arg), and CCA (Pro)]. B*070204 introduces a new codon at 57, CCT (Pro). Differing from B*0727 by five nucleotides at codons 77–83, the introduced amino acid sequence motif (NLRIALR) in B*0738 adds the Bw4 epitope. Within the B*07 group, the Bw4 epitope sequence in B*0738 is shared only with B*0736. Two cells were described with B*4812. The sequence of exon 2 of this new allele is identical to that found in B*4801 but exon 3 differs by five nucleotides encodins three different amino acids. These five nucleotides are shared with B*4802. The predicted haplotype carrying the new alleles is A*0222, B*4812,

Table 1 Cells and their the human leukocyte antigen (HLA) alleles

Cell	Race/ethnicity	HLA-A[a]	HLA-B[b]	HLA-C[a]	HLA-DR[a]	GenBank accession Number[c]
GN00433	Unknown	A*030101, *6801	B***070204,** *3503	NT[d]	NT	AY296125, AY296126
BY00054	Caucasoid	A*0301, *2403	B***0738,** *3508	Cw*070201, *0401	DRB1*1104,1501	AY562130, AY562131
NT00571	Caucasoid	A*01TUS, *24AGVK	B***0742**, *0801	Cw*07BJ, *07WTR	DRB1*03WHX, *15VYF	DQ007037, DQ007038
GN00434	Unknown	A*01CRY, *02GNF	B***0821,** *0702	Cw*070201, *07AG	DRB1*04DEX, *1301	AY296127, AY296128
NT00574	Caucasoid	A*01WUS, *3001	B***130202,** *5701	Cw*0602, *06BG	DRB1* 07YRE	DQ007041, DQ007042
NT00629	Caucasoid	A*02ANNS, *3001	B***130202**, *15010101	Cw*03FJC, *06AJB	04NJV, 07APA	DQ334731, DQ334732
GN00416	Unknown	A*02, *3401	B***1312, ***380202	Cw*0403, *07020101/02/03	NT	AY428806, AY428807
GN00425	Unknown	A*30, *33	B***1575,** *440302	NT	NT	AY178185, AY178186
GN00427	Unknown	A*30	B***1575**, *1302	NT	NT	AY178189, AY178190
NT00523	African-American	A*02YAH, *30GSH	B***1598,** *510101	Cw*0202, *02BD	DRB1*07APA, *11WXE	AY877249, AY877250
NT00518	African-American	A*3402, *68XX	B***1599,** *8101	Cw*030402, 0804	DRB1*15JST, *1001	AY877255, AY877256
GN00360	Caucasoid	A*01, *02	B***270507,** *0801	Cw*020202, *07WTR	NT	AF266521[e], AF266522, AY700219
NT00514	Caucasoid	A*01XX, *680101	B***2728, ***08DKG	NT	DRB1*03KAJ, *13KBC	AY877253, AY877254
NT00573	African-American	A*02ADVV, *02VFS	B***350104, ***530101	Cw*04ZFD, *1601	DRB1*07APA, *08CKE	DQ007039, DQ007040
NT00565	Unknown	A*68	B***3558,** *3521	Cw*04KGB	DRB1*04YK,*04DJX	AY907708, AY907709
NT00566	American-Indian	A*24AGUG, *26STB	B***3811, *** 390602	Cw*07BJ, *12NP	DRB1*04ADJ, *14ACZ	AY956752, AY956753
NT00509	Unknown	A*02GNF, *030101	B***3931,** *2702	Cw*020202, *120301	DRB1*110101, *070101	AY607032, AY607033
BY00055	Caucasoid	A*03	B***3932, ***1801	Cw*0501/03, *0702	DRB1*03, *04	AY607030, AY607031
GN00423	Unknown	A*24	B***4045, ***1535	Cw*030401/03, *0702	DRB1*12, *14	AY178187, AY178188
NT00568	Unknown	A*68AEBA, *6901	B***4107,** *1504	Cw* 010201, 17MN	DRB1*07MT, *14BF	AY935260, AY935261
BY00048	Unknown	A*02PSD, *030101G1/0308	B***420501,** *400101	NT	DRB1*150101, *0404	AY217666, AY217667
NT00521	Caucasoid-Hispanic	A*0222, *29MS	B***4812,** *13AB	Cw*0602, *0803	DRB1*07MT, *08BYC	AY874083, AY874084
NT00569	Caucasoid	A*02ARBS, *02ARBT	B***4812,** *3543	Cw*01AEV, *08CF	DRB1*0901	AY956750, AY956751
NT00525	Oriental	A*02XSG, *11XSH	B***510106, ***5401	Cw*01TF, *14BC	DRB1*08YMJ, *1403	AY877251, AY877252
BY00104	Caucasoid	A*0101, *0201	B***5520,** *150101	Cw*0401, *0701	DRB1*0701, *1302	AY504809, AY504810,
NT00572	Unknown	A*01YAG, *03BKS	B***5520**, *350101	Cw*04TSR, *07WTR	DRB1*01RY, *13GPE	DQ120786, DQ120787
NT00595	Caucasoid	A*03AD, A*29WWP	B***5616**, *15010101	Cw*01BG, *03FJC	DRB1*04NJV, *0901	DQ0965573, DQ096574

[a] HLA-A, -C, and -DRB1 were assigned primarily by probe-based typing. Letter codes indicate alternative alleles. The alleles included are described at the bioinformatics.nmdp.org web site.

[b] Novel allele is in bold type. The names have been officially assigned by the World Health Organization Nomenclature Committee (2). Alleles were assigned by DNA sequencing.

[c] Accession number of novel allele (exon 2 and exon 3).

[d] NT, not tested.

[e] Accession number of novel allele (exon 1).

Cw*0803. B*5520 is a recombinant allele: the sequence of exons 1 and 2 is shared with B*550101 and exon 3 with one nucleotide (and amino acid) substitution is shared with B*4204. Found in two cells, predicted haplotype carrying the new allele is A*0101, B*5520, DRB1*1302. Two haplotypes carrying new alleles are A*3001, B*130202, Cw*0602, DRB1*0701,0703,0705,0707 and A*30, B*1575.

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Table 2 Description of novel sequences

Novel allele[a]	Most homologous allele[b]	Difference (number of nucleotides)	Codon changes[c]	Amino acid changes	Alternative nucleotide (amino acid substitution) found at the altered codon and number of occurrences[d]
B*070204	B*070201	1	57 CC**G** to CC**T**	Silent	57: CCG (P) 723, CGG (R) 1, CCA (P) 1, **CCT (P)** 1
B*0738	B*0727	5	67 T**C**C to T**T**C	67 S to F	67: TCC (S) 303, **TTC (F)** 166, TAC (Y) 122, TGC (C) 105, ATG (M) 28, TCG (S) 2
			77 **G**AC to **A**AC	77 D to N	77: AGC (S) 492, **AAC (N)** 197, GAC (D) 35, GGC (G) 1, TGC (C) 1
			80 A**C**C to A**T**C	80 T to I	80: AAC (N) 488, **ATC (I)** 119, ACC (T) 118, AAG (K) 1
			81 **CT**G to **GC**G	81 L to A	81: CTG (L) 534, **GCG (A)** 191, CCG (P) 1
B*0742	B*070201	1	181 **C**GC to **A**GC	181 R to S	181: CGC (R) 721, **AGC (S)** 1, CAC (H) 1
B*0821	B*0811	2	163 **GC**G to **CT**G	163 A to L	163: **CTG (L)** 348, GAG (E) 197, ACG (T) 178, GCG (A) 1, GGG (G) 1, ATG (M) 1
B*130202	B*130201	1	39 GA**C** to GA**T**	39 silent	39: GAC (D) 724, AAC (N) 1, **GAT (D) 1**
B*1312	B*1301	1	163 G**A**G to G**G**G	163 E to G	163: CTG (L) 348, GAG (E) 197, ACG (T) 178, GCG (A) 1, **GGG (G)** 1, ATG (M) 1
B*1575	B*150102	2	121 AA**A** to AA**G**	121 silent	121: **AAG (K)** 659, AAA (K) 67
			152 **G**AG to **C**AG	152 E to Q	152: GTG (V) 448, GAG (E) 274, GCG (A) 1, **CAG (Q)** 1, ACG (T) 1, GTC (V) 1
B*1598	B*1503	1	43 C**C**G to C**T**G	43 P to L	43: CCG (P) 721, **CTG (L)** 2, CCA (P) 1, CAG (Q) 1, CCC (P) 1
B*1599	B*1510	1	156 C**T**G to C**G**G	156 L to R	156: CTG (L) 485, GAC (N) 94, **CGG (R)** 77, TGG (W) 69, GAA (E) 1
B*270507	B*270502	1	161 GA**G** to GA**A**	161 silent	161: GAG (E) 725, **GAA** (E) 1
B*2728	B*270502	3	163 **GA**G to **AC**G	163 E to T	163: CTG (L) 348, GAG (E) 197, **ACG (T)** 178, GCG (A) 1, GGG (G) 1, ATG (M) 1
			171 **T**AC to **C**AC	171 Y to H	171: TAC (Y) 631, **CAC (H)** 95
B*350104	B*350101	1	99 TA**T** to TA**C**	99 silent	99: **TAC (Y)** 399, TAT (Y) 302, TTT (F) 12, TCT (S) 12, TGC (C) 1
B*3558	B*3505	1	152 G**T**G to G**A**G	152 V to E	152: GTG (V) 448, **GAG (E)** 274, GCG (A) 1, CAG (Q) 1, ACG (T) 1, GTC (V) 1
B*3811	B*380101	2	52 **A**T**A** to **G**T**G**	52 I to V	52: ATA (I) 715, **GTG (V)** 11
B*3931	B*39010101	1	9 **T**AC to **C**AC	9 Y to H	9: TAC (Y) 533, **CAC (H)** 164, GAC (D) 28
B*3932	B*39010101	3	163 **AC**G to **CT**G	163 T to L	163: **CTG (L)** 348, GAG (E) 197, ACG (T) 178, GCG (A) 1, GGG (G) 1, ATG (M) 1
			171 **T**AC to **C**AC	171 Y to H	171: TAC (Y) 631, **CAC (H)** 95
B*4045	B*400102	2	97 AG**G** to AG**C**	97 R to S	97: AGG (R) 398, **AGC (S)** 144, ACG (T) 125, AAT (N) 35, GTG (V) 15, TGG (W) 8, ATG (M) 1
			147 T**T**G to T**G**G	147 L to W	147: **TGG (W)** 684, TTG (L) 42
B*4107	B*4101	1	103 **G**TG to **C**TG	103 V to L	103: GTG (V) 556, **CTG (L)** 169, ATG (M) 1
B*420501	B*420101	2	178 A**C**G to A**A**G	178 T to K	178: ACG (T) 621, **AAG (K)** 102, ATG (M) 1
			182 GC**G** to GC**T**	182 silent	182: GCG (A) 618, **GCT (A)** 102, TCT (S) 1
B*4812	B*4801	5	131 **C**GC to **A**GC	131 R to S	131: **AGC (S)** 531, CGC (R) 195
			135 GC**C** to GC**G**	135 silent	135: **GCG (A)** 534, GCC (A) 192
			138 AC**G** to AC**C**	138 silent	138: **ACC (T)** 406, ACG (T) 317, ACA (T) 2, AAG (K) 1
			143 **T**CC to **A**CC	143 S to T	143: **ACC (T)** 680, TCC (S) 44, ACT (T) 1, ATC (I) 1
			147 T**T**G to T**G**G	147 L to W	147: **TGG (W)** 684, TTG (L) 42
B*510106	B*510101	1	69 AC**C** to AC**T**	69 silent	69: ACC (T) 548, GCC (A) 165, CGC (R) 10, GGC (G) 1, **ACT (T)** 1, ATC (I) 1
B*5520	B*4204	5	11 **T**CC to **G**CC	11 S to A	11: **GCC (A)** 493, TCC (S) 230, GCT (A) 2
			12 **G**TG to **A**TG	12 V to M	12: **ATG (M)** 483, GTG (V) 242
			24 **T**CA to **G**CA	24 S to A	24: **GCA (A)** 305, TCA (S) 224, ACC (T) 194, TCC (S) 1, CCC (P) 1
			31 AC**C** to AC**G**	31 silent	31: ACC (T) 374, **ACG (T)** 351
			103 **G**TG to **C**TG	103 V to L	103: GTG (V) 556, **CTG (L)** 169, ATG (M) 1
B*5616	B*5601	1	24 **G**CA to **T**CA	24 A to S	24: GCA (A) 305, **TCA (S)** 224, ACC (T) 194, TCC (S) 1, CCC (P) 1

[a] Exons 2 and 3 of the human leukocyte antigen (HLA)-B alleles were characterized by DNA sequencing. In some cases, the HLA-B alleles were amplified separately from genomic DNA with group-specific HLA-B primers and identified using sequence-based typing as previously described (3, 4). The polymerase chain reaction (PCR)-amplified HLA-B alleles in these samples were sequenced using an ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction kit and a 3730 xl sequencer (Applied Biosystems, Foster City, CA, USA). Amplicons from samples with a novel allele that could not be isolated individually by group-specific PCR were ligated into the pCR2.1-TOPO vector and cloned (HPT TOPO TA cloning kit, Invitrogen, Carlsbad, CA). Sequencing of several random bacterial colonies determined the sequence of the allele. Both the HLA-B alleles from a cell were also sequenced together using previously described primers by our group (3) and (4, 5) to provide an additional confirmation of the two alleles present.

[b] Most homologous sequence obtained from IMGT/HLA sequence database (www.ebi.ac.uk/imgt/hla/).

[c] Numbering from the first codon of the mature protein. The altered codon(s) number and sequence of the most homologous allele compared with the novel allele is listed second. The nucleotide change(s) is in bold.

[d] The alternative sequences present at the altered codon are listed with the number of occurrences. For example, allele B*070204 differs from B*070201 at codon 57. At this codon, in all HLA-B alleles, nucleotide sequences are found: CCG encoding proline in 723 HLA-B alleles, CGG encoding arginine in one allele, CCA encoding proline in one allele, and CCT encoding proline in one allele. The sequence found in the novel allele is in bold. The information was obtained from IMGT/HLA sequence database release Version Report – 2.12 (01/2006) at www.ebi.ac.uk/imgt/hla/.

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