SIXTEEN NOVEL AND NINE NEWLY CONFIRMED ALLELES OF THE HLA-A, -B, -C, AND -DPA1 LOCI IDENTIFIED DURING ROUTINE CLINICAL

Alison J. Gareau, Ana Lazaro-Shiben, Kristin Gay, Marlee Folckomer, Maria Bettinotti
Immunogenetics Laboratory, Johns Hopkins University, School of Medicine

Abstract

Aim: Sixteen novel alleles and 9 confirmatory sequences for previously submitted alleles were identified over the course of one year of routine clinical testing.

Methods: HLA typing was performed at high-resolution using the hybrid-capture-based AlloSeq_Tx17 kit from CareDx for next generation sequencing (NGS). The alleles were initially identified as novel when the NGS sequencing results showed well-defined and distinct mismatches to all the HLA allelic sequences for the locus in question. The novel alleles were confirmed with repeat sequencing or segregation within a family.

Results: Eleven of the 25 alleles were found in African-American individuals (44%), 9 (36%) in Caucasian individuals, 1 (0.25%) in an Asian individual, and 4 (1%) in individuals of unknown race. Twenty-one (84%) out of 25 alleles were single substitution variants when compared with their most similar allele, with 5 of these (23%) being silent substitutions with no change in the amino acid. Four (16%) out of 25 showed two or more substitutions. HLA-A*29:160 was observed three times within one family. DPA1*01:03:31, *01:58:01 and *02:01:16 each appeared twice in one family. Conforming to the linkage disequilibrium (LD) between HLA-B and C, all new HLA-B alleles were associated with the HLA-C allele linked to the ancestral HLA-B allele. For example, HLA-B*15:623 showed linkage with C*04:03 (B*15:21, C*04:03 - AFA 0.00341 rank 60);B*14:109 with C*08:02 (B*14:02, C*08:02 - AFA 0.02076 rank 13); B*07:456 with C*07:02, (B*07:02, C*07:02 - Cau 0.13782 rank 1); B*42:32 with C*17:01 (B*42:01, C*17:01 - AFA 0.05318, rank 4); B*39:177 with C*12:03 (B*39:01, C*12:03 - Cau 0.006 rank 31) and B*44:545 with C*04:01 (B*44:03, C*04:01 - Cau 0.014 rank 19).

Conclusion: Approximately one-third (36%) of these alleles were discovered in patients awaiting bone marrow transplantation or in potential donors and 44% in candidates for solid organ transplantation. Allele-level resolution typing is important not only for bone marrow transplantation but also in the solid organ transplantation setting to investigate eplet-based matching and to supplement current antibody analysis practices. It is very important to continue identifying and publishing these allele sequences in collaboration with the IMGT and the IPD-IMGT/HLA Database for the most robust assignment of alleles.

Novel Alleles/ Confirmatory Hybrid-Capture-based AlloSeq_Tx17 CareDx

Extraction

Fragmentation Indexing Enrichment

Size Selection Purification Pooling

Probe Hybridization Capture Post Enrichment

Purification

Dilution 4nM Denaturation Final Pooled Loading

Novel & Confirmatory Alleles

Novel Allele	Most Homologous Allele	Difference (number of nucleotides)	Codons changes	Amino acid changes
A*29:160 Conf	**A***29:02:01:01	1Ex3	**A**GC to **G**GC	205 S to G
A*03:443	**A***03:01:01:01	1Ex2	C**G**C->C**A**C	17R to H
		1 Intron 6	2606 C->T	
A*03:420 Conf	**A***03:01:01:01	1Ex1	**G**TC->**A**TC	(-22) V to I
A*32:01:51	**A***32:01:01:01	1Ex5	ATC->ATT	287 Silent I
A*33:XX	**A***33:03:01	1Ex4	ACC->AC**A**	200 Silent T
		1 5'UTR	(-196) T->C	
A*02:987 Conf	**A***02:01:01:01	1Ex4	TG**C**->TG**G**	259 C to W
A*02:1054	**A***02:17:02:01	1Ex2	CA**G**->CA**C**	87 Q to H
		1 Intron 5	1269 A->C	
B*15:623	**B***15:21:01:01	1Ex 2	**G**TG->**C**TG	103V to L
		1Ex2	CC**G**->CC**C**	105 Silent P
		1Ex2	**T**AT->CAT	113 Y to H
		1Ex2	AC**G**->AC**C**	138 Silent T
		1Ex2	G**A**G->G**T**G	152 E-to V
B*14:109	**B***14:02:01:01	1Ex5	A**G**G->A**A**G	309 to K
B*07:456	**B***07:02:01:01	Lider peptide	(-16) **G**TC->**C**TC	(-16) V to L
B*42:32	**B***42:01:01:01	1Ex3	T**A**C->T**C**C	116 Y to S
B*39:177 conf	**B***39:01:01	1Ex6	T**A**C->T**G**C	320 Y to C
		Intron 5	2407 T->C	
B*44:545	**B***44:03:01:01	1Ex2	**G**CG->TCG	81 A to S
		1 3'UTR	3412 G->T	
		1 3'UTR	3472 C->T	
		1 3'UTR	3507 A->T	
B*53:01:28	**B***53:01:01:01	1Ex4	CC**C**->CC**T**	195 Silent P
B*15:632	**B***15:01:01:01	1Ex6	G**C**G->G**T**G	324 A toV
C*03:596	**C***03:02:02:05	1Ex3	C**T**C->C**A**T	110 L to H
DPA1*01:58:01:01 Conf	**DPA1***01:33	1Ex2	**A**CC->**G**CC	83 T toA
		1Ex2	AA**C**->AA**T**	84 Silent N
DPA1*02:01:16 Conf	**DPA1***02:01:01:01	1Ex3	AC**C**->AC**G**	90 Silent T
		1Ex3	AA**C**->AA**T**	118 silent N
		1Ex3	CC**A**->CC**G**	127 Silent P
DPA1*02:64	**DPA1***02:02:02:01	1Ex1	**A**TG->ACG	(-31.2) M toT
DPA1*02:43 Conf	**DPB1***02:01:01:01	1Ex1	AAT->AGT	84 N to S
DPA1*01:87Q Conf	**DPA1***01:03:01:01	1Ex1	**A**TG->AC**G**	(-31.2) M toT
DPA1*01:03:31 Conf	**DPA1***01:03:01:01	1Ex3	TAC->TA**T**	150 Silent Y
DPA1*01:100	**DPA1***01:03:01:01	1Ex2	GC**G**->GT**G**	3 A to V
DPA1*02:22:02	**DPA1***02:22	1 Ex2	GG**A**->GG**G**	20 Silent G
DPA1*01:103	**DPA1***01:03:01:05	1Ex2	GC**G**->G**A**G	10 A to E

Extended Haplotype of the New Allele

Cell	Pt Type	Race/Ethnicity	HLA-A	HLA-B	HLA-C	HIA-DRB1	DRB3/4/5	HLA-DQA1	HLA-DQB	HLA-DPA1	HLA-DPB1
JH0001	BM	Unknown	**24:07:01**	***15:623***	**04:03:01**	**15:02:01**	**B5 01:01:01**	**01:02:01**	**06:01:01**	**02:02:02**	**31:01P**
JH0002	BMFam	African American	**30:01:01**	***14:109***	**08:02:01**	**03:01:10,**	**B302:02:01**	**05:01:01**	**02:01:01**	**01:03:01**	**02:01P**
JH0008	BMFam	Unknown	**23:01:01**	**07:02:01**	**15:05:02**	**03:01:01**	**B302:02:01**	**05:01:01**	**02:01:01**	***02:01:16***	**13:01P**
JH0012	BMFam	Caucasian	**24:02:01**	**44:02:01**	**05:01:01**	**04:01:01**	**B401:03:01**	**03:03:01**	**03:01:01**	***01:03:31***	**02:01P**
JH0014	BM	Caucasian	**30:01:01**	**42:01:01**	**17:01:01**	**03:01:01**	**B302:02:01**	**05:01:01**	**02:01:01**	***02:22:02***	**01:01:01**
JH0015	BMFam	African American	***29:160***	**53:01:01**	**04:01:01**	**13:02:01**	**B303:01:01**	**01:02:01**	**06:09:01**	**02:01:01**	**01:01P**
JH0021	BMFam	Unknown	***02:1054***	**35:12:01**	**04:01:01**	**14:02:01**	**B301:01:02**	**05:03:01**	**03:01:01**	**01:03:01**	**04:02P**
JH0023	BM	Caucasian	**01:01:01**	***15:632***	**03:04:01**	**01:01:01**		**01:01:01**	**05:01:01**	**01:03:01**	**03:01P**

HLA-B-C LD from the ancestral HLA-B/C association

HLA-B	HLA-C	HLA-B	HLA-C	EUR_freq	EUR_rank	AFA_freq	AFA_rank	API_freq	API_rank
07:456	03:04:01	**0702g**	**03:04**	0.00006	229	0.00046	138	0.00000	NA
44:545	04:01:01	**4403**	**0401g**	0.01434	19	0.02888	8	0.00202	73
15:623	04:03:01	**1521**	**0403**	0.00000	NA	0.00000	NA	0.00341	60
14:109	08:02:01	**1402**	**0802**	0.03038	9	0.02076	13	0.00114	108
39:177	12:03:01	**3901g**	**1203**	0.00616	31	0.00187	76	0.00029	173
42:32	17:01:01	**4201**	**1701g**	0.00000	NA	0.05318	4	0.00000	NA
15:632	03:04:01	**15:01:01:01**	**03:04**	0.02436	11	0.00291	57	0.00159	82
58:01:01	**03:596**	**5801g**	**0302**	0.00146	53	0.01039	27	0.05543	2

JOHNS HOPKINS MEDICINE

Conclusions

- ❑ Sixteen novel alleles and 9 confirmatory sequences from previously submitted alleles were identified over the course of one year of routine clinical testing. HLA typing was performed at high-resolution using next generation sequencing (NGS).
- ❑ Twenty-one (84%) out of 25 alleles were single substitution variants when compared with their most similar allele, with 5 of these (23%) being silent substitutions with no change in the amino acid. Four (16%) out of 25 showed two or more substitutions.
- ❑ It is very important to continue identifying and publishing new allele sequences in collaboration with the IMGT and the IPD-IMGT/HLA Database for a most robust assignment of alleles.