EXTENDED HAPLOTYPES BETWEEN CLASSICAL AND NON-CLASSICAL HLA GENES IN A MULTIPLE FAMILY STUDY

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Abstract

Aim: In this study, we identified 180 unique extended haplotypes in 50 families using NGS high-resolution HLA typing.

***Methods*:** HLA typing was performed using the AlloSeqTx17 hybrid capture-based assay (CareDx). Samples were typed at 17 loci, including 11 classical loci and the non-classical HLA-E, -F, -G, -H, -MICA, and -MICB loci.

Results: The composition of the families studied was 68% Caucasian, 17% African American, 6% Asian, 1% Hispanic, and 8% unknown. We observed that only a minor subset of the non-classical loci alleles described in the IPD-IMGT/HLA Database was represented in this population. HLA-H is a pseudogene located at 55 kilobase pairs from the telomeric end of HLA-A, with these genes sharing a high degree of similarity (Jordier, 2020). We observed the following HLA-A~HLA-H haplotypes: A1 (01:01:01) with H*02:01:01, A2 (02:01:01:01, 02:09:01) with H*01:01:01; A9 (23:01:01, 23:17:01, 24:02:01) with a deletion of HLA-H; A10 (25:01:01, 26:01:01, 66:01:01) with H*01:02:01; A3 (03:01:01, 03:02:01) with H*02:04:01; A11 (11:01:01) with H*02:07:01; A28 (68:01:01, 68:01:02) with H*02:05:01; A29 (29:01:01, 29:02:01) with H*02:02:01; A32 (32:01:01) with H*02:03:02, and A33 (33:01:01) with H*02:08:01. We looked at extended haplotypes within the alpha block (HLA-A~HLA-F~HLA-G~HLA-H), finding statistically significant LD for the following haplotypes: A*02:01:01-G*01:01:01-H*01:01:01 (t=8); A*03:01:01-G*01:01:01-H*02:04:01 (t=5); A*23:01:01/24:02:01-F*01:03:01-G*01:04:04 - H*DEL (t= 4); A*25:01:01/26:01:01-F*01:01:01-G*01:01:02-H*01:02:01 (t= 6). In the beta block, HLA-B~C~MICA~MICB were seen in LD for the following combinations: B*07:02:01-C*07:02:01-MICA*08:04-MICB*04:01:01 (t=4.5); B*08:01:01-C*07:01:01-MICA*008:01-MICB*008:01 (t=3.29) and B*44:02:01:01-C*05:01:01-MICA*008:01-MICB*005:02 (t=4). [A 2X2 table was used to estimate LD using t values: t=Δij/SEΔij; if t > 2 in its absolute value, the existence of statistically significant LD is considered, (SE=Standard Error)].

Conclusion: In summary, this study demonstrates a strong association between the classical and non-classical HLA alleles in concordance with previously published data. Analysis of a large number of unique haplotypes will be required to better characterize the classical and non-classical HLA linkage disequilibrium and their role in both bone marrow and solid organ transplantation.

Chromosome 6

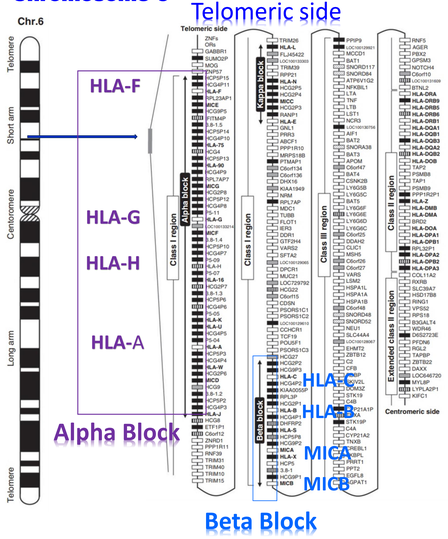

Figure 1 Gene map of the human leukocyte antigen (HLA) region
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HLA-A-G-H Haplotype Ancestral evolution

HLA-G	HLA –H	HLA-A Allele	HLA-A Linage
G*01:01:01	H*01:01:01	A*02:01:01 A*02:09:01	A2
G*01:03:01	H*01:03:01	A*02:05:01 A*02:02:01	A2
G*01:01:02	H*01:02:01	A*25:01:01 A*26:01:01	A10
G*01:01:19	H*01:02:01	A*66:01:01	A10
G*01:01:02 G*01:06:01	H*02:01:01 H*02:01:01	A*01:01:01	A1
G*01:01:01	H*02:02:01	A*29:01:01 A*29:02:01	A29
G*01:01:01	H*02:04:01	A*03:01:01 A*03:02:01	A3
G*01:01:02	H*02:05:01	A*68:01:01 A*68:01:02	A28
G*01:05N G*01:01:01	H*02:05:01 H*01:01:02/01:04	A*30:01:01 A*30:02:01	
G*01:01:03	H*02:07	A*11:01:01	A11
G*01:01:09	H*02:11:01	A*34:02:01	A34
G*01:04	H Deleted	A*23:01:01 A*23:17:01 A*24:02:01	A9

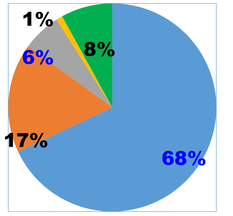
BETA Block: Linkage Disequilibrium Between HLA B and Non Classical HLA allele

HLA-B-MICA-MICAB	Delta	Linkage Disequilibrium > = 2 LD
HLA-B*07:02:01-MICA*08:04-MICB*04:01:01	0.054	4.5
HLA-B*08:01:01-MICA*008:01-MICB*008:01	0.031	3.29
HLA-B*44:02:01:01-MICA*008:01-MICB*005:02	0.03	3.3

ALPHA Block : HLA -A in LD with the Non Classical HLA alleles

HLA-A-G-H	Delta	Linkage Disequilibrium > = 2 LD
HLA-A*02:01:01-G*01:01:01-H*01:01:01	0.14	7.7
HLA-A*03:01:01-G*01:01:01-H*02:04:01	0.08	5
HLA-A*11:01:01-G*01:01:03-H*02:07:01	0.03	3.3
HLA-A-F-G-H		
HLA-A*23:01:01-F*01:03:01-G*01:04:04-H*DEL	0.023	2.9
HLA-A*25:01:01/26:01:01-F*01:01:01-G*01:01:02-H*01:02:01	0.053	6
HLA-A*29:02:01-F*01:01:01-G*01:01:01-H*02:02:01	0.077	3.3
HLA-A*68:01:01-F*01:01:01-G*01:01:02-H*02:05:01	0.028	2.15
HLA-A-G-H		
HLA-A*24:02:01-G*01:04:01-H*DEL	0.018	2.4
HLA-A-H		
HLA-A*23:01:01/24:02:01 -H* DEL	0046	4.18
HLA-A*24:02:01-G*01:04:01-H*DEL	0.018	2.4
HLA-A*32:01:01-H*02:03:02	0.016	2.66

Ethnic Composition of the 50 families



Conclusions

- The genetic diversity of the non-classical HLA loci and linkage disequilibrium (LD) with classical HLA loci was explored on 180 unique extended haplotypes from 50 families
- High-resolution HLA typing was performed with the AlloSeqTx17 hybrid capture-based assay (CareDx). Samples were typed at 17 loci, including 11 classical loci and the non-classical HLA-E, -F, -G, -H, -MICA, and –MICB
- We looked at extended haplotypes within the alpha block (HLA-A~HLA-H~HLA-G~HLA-F), finding statistically significant LD for the following haplotypes: A*02:01:01-G*01:01:01-H*01:01:01; A*03:01:01-G*01:01:01-H*02:04:01; A*23:01:01/24:02:01-F*01:03:01-G*01:04:04 - H*DEL; A*25:01:01/26:01:01-F*01:01:01-G*01:01:02-H*01:02:01.
- It is important to characterize the classical and non-classical HLA linkage disequilibrium and further investigate clinical implications in both bone marrow and solid organ transplantation.