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Research article

HLA Haplotypes In 250 Families: The Baylor Laboratory Results And A Perspective On A Core NGS Testing Model For The 17th International HLA And Immunogenetics Workshop

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ABSTRACT

Since their inception, the International HLA & Immunogenetics Workshops (IHIW) served as a collaborative platform for exchange of specimens, reference materials, experiences and best practices. In this report we present a subset of the results of human leukocyte antigen (HLA) haplotypes in families tested by next generation sequencing (NGS) under the 17th IHIW. We characterized 961 haplotypes in 921 subjects belonging to 250 families from 8 countries (Argentina, Austria, Egypt, Jamaica, Germany, Greece, Kuwait, and Switzerland). These samples were tested in a single core laboratory in a high throughput fashion using 6 different reagents/software platforms. Families tested included patients evaluated clinically as transplant recipients (kidney and hematopoietic cell transplant) and their respective family members. We identified 486 HLA alleles at the following loci HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, -DPB1 (77, 115, 68, 69, 10, 6, 4, 44, 31, 20 and 42 alleles, respectively). We also identified nine novel alleles with polymorphisms in coding regions. This approach of testing samples from multiple laboratories across the world in different stages of technology implementation in a single core laboratory may be useful for future international workshops. Although data presented may not be reflective of allele and haplotype frequencies in the countries to which the families belong, they represent an extensive collection of 3rd and 4th field resolution level 11-locus haplotype associations of 486 alleles identified in families from 8 countries.

Abbreviations: CWD, Common and Well Documented; GL, Genotype List; HLA, Human Leukocyte Antigen; IHIW, International HLA and Immunogenetics Workshop; IMGT, ImMunoGeneTics; IPD, ImmunoPolymorphism Database; LD, Linkage Disequilibrium; MHC, Major Histocompatibility Complex; NGS, Next Generation Sequencing

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1. Introduction

In 1964, Dr. Bernard Amos organized the first international human leukocyte antigen (HLA) & immunogenetics workshop (IHIW) in his laboratory at Duke University [1]. Since their inception, these workshops have been the international forums where histocompatibility investigators and practitioners from all over the world convene to compare methods and to share reagents and specimens. The 17th International HLA and Immunogenetics Workshop (17th IHIW) was held September 6–10, 2017, in Pacific Grove, CA with a major emphasis on the applications of next generation sequencing (NGS) technology in histocompatibility and immunogenetics.

The Major Histocompatibility Complex (MHC) region spans approximately 3.8 megabases (Mb) of the short arm of chromosome 6 at 6p21.3 and includes over 269 loci [2,3]. The Immune Polymorphism Database (IPD)-IMGT/HLA is part of the international ImMunoGeneTics project (IMGT) [4]. It is a specialized database that houses sequences of the MHC and the official sequences named by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System. The 3.34.0 release of IPD-IMGT/HLA database (10/2018) included 20088 alleles of HLA class I genes (A, B, C, E, F, G) and HLA class I pseudogenes (H, J, K, L, N, P, S, T, U, V, W, Y), and HLA Class II genes and pseudogenes (DRA, DRB, DQA1, DQB1, DPA1, DPA2, DPB1, DPB2, DMA, DMB, DOA, DOB). These thousands of alleles are not inherited at random. Rather, these alleles are inherited in linked strings corresponding to maternal and paternal chromosomes, known as HLA haplotypes [5]. Family pedigree analysis has shown that recombination occurs at specific locations within the MHC, leading to a structure of four major genomic blocks. Dawkins and colleagues referred to these blocks as the alpha, beta, gamma, and delta blocks [6]. The alpha block contains HLA-A, the beta block contains HLA-C and HLA-B, the gamma block contains the complement proteins C2, C4, and factor B (Bf), and the delta block contains HLA-DR and HLA-DQ [7]. A unique characteristic of the MHC is the extensive linkage disequilibrium (LD) observed among distant genomic regions. The LD is stronger among loci within the same MHC gene block, e.g., the B and C loci in the beta block and the DR and DQ loci in the delta blocks [6]. An exception is the weak LD between the HLA-DPB1 and the DQB1 loci due to the presence a hot spot of recombination between the two loci [8]. According to this model, HLA-DP loci are not included in the delta block as a block is defined as two or more loci without an intervening hotspot of recombination. Although the LD between the 2 HLA-DP loci (DPA1 & DPB1) has been reported to be fairly strong, the association between these loci and the rest of class II loci is less predictable [9–13].

HLA haplotypes are defined by the constellations of alleles inherited together on the same chromosome. These allele combinations are typically conserved through generations by the virtue of limited recombination among different MHC loci [14]. Allele and haplotype frequencies are fairly stable in a given ethnic group but vary among different racial and ethnic groups [15]. Phasing of haplotypes is classically determined by Mendelian segregation through family studies. In samples of unrelated individuals, bioinformatics approaches such as the Expectation Maximization (EM) algorithm can be used to make statistical inference of allele associations within haplotypes. Such algorithms can resolve ambiguous genotypes and multiple allele name resolutions by exploiting population LD information and generating population-specific haplotype frequencies [16–19]. These frequencies are in turn used by imputation algorithms to resolve allele and phase ambiguities in HLA genotype data using LD information embedded in population haplotype frequencies [20–22]. However, family pedigrees remain the gold standard for studying HLA haplotype segregation. This information is critical when studying haplotype associations, especially among populations where not enough members of the population have been genotyped and in mixed populations where bioinformatic imputation algorithms are less reliable compared to populations where sizable number of individuals have been studied [20].

It was by design that NGS was the main focus of the 17th IHIW for a myriad of reasons including accuracy, high throughput, and lower cost. The merits of NGS inspired the editors of the Nature Methods journal to name it the inaugural “method of the year” in 2008, starting a tradition of bestowing this status on one method every year thereafter [23]. In keeping with the long-standing tradition of the international workshop, laboratories from different parts of the world were encouraged to participate. The goal of this project was to conduct family-based studies by NGS of HLA genes to determine haplotype segregation and compare their distribution among multiple populations. This project also compiled a large library of sequencing raw data (FASTQ) files to be used in the future for cross-platform validation. This report presents a subset of the project results involving families that were tested by NGS in the Baylor University Medical Center Transplant Immunology Laboratory. Participating laboratories from different parts of the world contributed samples that were initially tested in the context of clinical evaluation of transplant recipients and their family members. These samples may not necessarily represent the populations of these countries at large. We also describe a core laboratory model for the IHIW testing, from the perspective of the Baylor University Medical Center Transplant Immunology Laboratory.

2. Methods

2.1. Sample collection

Eight laboratories participating in this project submitted samples from Argentina, Austria, Egypt, Jamaica, Germany, Greece, Kuwait, and Switzerland. Participants contributed samples from family quartets (n = 128) consisting of two parents and at least two non-HLA identical children and family trios (n = 122) consisting of one parent and at least two non-HLA identical children or two parents and one biological child. All members of the families were previously HLA typed. Participating laboratories submitted 2 μg DNA/sample from all members of the included families.

2.2. Typing method

All individuals were originally HLA typed in the participating laboratories and results were used to establish family pedigrees. There was no requirement of a particular method of typing or number of HLA loci by the participating laboratories provided that family pedigrees could be established. The core laboratory typed all DNA samples using NGS reagents from one of six vendors (Gen Dx Products, Inc; Illumina, Immucor, GenDx, Omixon, Scisco Genetics and One Lambda (Thermo Fisher Scientific)) and sequenced using MiSeq instruments (Illumina, San Diego, CA). HLA genotypes were assigned using the corresponding NGS genotyping software with IPD-IMGT/HLA Database version 3.25.0.

2.3. Quality assurance measures

Prior to testing workshop samples, the core laboratory participated in the quality control project of NGS HLA genotyping for the 17th IHIW that required a blind testing of 96 reference samples with > 95% concordance rate [24]. In addition, we considered the family studies to be an additional quality measure, as all alleles identified on a haplotype needed to be accounted for in different family members.

2.4. HLA genes and regions sequenced

Eleven loci (HLA-A, C, B, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1) were tested by NGS. Specific genomic regions interrogated by each kit used in the project are listed in Table 1.

2.5. Data collection

All samples submitted for testing by participating laboratories were registered in the 17th IHIW database, including the relationship of the family members and pedigree analysis of each family. Each sample and each family were uniquely identified by an ID automatically generated by the database. [25]. HLA types were imported in GL string format which was developed as a standard software-consumable HLA genotype data format [26,27]. This format is being widely adopted in the immunogenetics community and the advantages of using it have been described previously [25,28–31]. Automated tools integrated in the database facilitated the conversion of data output from different analysis software packages into the desired 17IHIW database-compatible format.

The Baylor laboratory uploaded HLA typing results and FASTQ files, and all data were converted to a standardized format that is described elsewhere [25]. When 4th field ambiguities could not be resolved, results are reported in the manuscript at the 3rd field resolution with the string of ambiguous alleles in the Supplemental Tables.

2.6. Haplotype assignment and bioinformatics analysis

Eleven-locus haplotypes were built from families using the HaplObserve software package that can be downloaded from the IHIW GitHub repository (https://github.com/ihiw/haplObserve) [32]. The software builds haplotypes using allele segregation within a nuclear family. When unambiguous haplotypes were not built using allele segregation in a family due to the absence of informative genotypes for *HLA-C~HLA-B* and *HLA-DRB3/4/5~HLA-DRB1~HLA-DQB1*, we used HLAHapV software to impute the haplotypes [33]. HLAHapV was developed to automate the application of allele prevalence and reference haplotype frequency data for calculating the likelihood of haplotype combinations. We used haplotype frequency tables of the National Marrow Donor Program of the United States (NMDP) as reference tables for HLAHapV [16]. The HLAHapV package and its instructions can be obtained from GitHub (https://github.com/nmdp-bioinformatics/ImmunogeneticDataTools). The detailed procedure for this process is described elsewhere [32]. Eleven-locus haplotypes were then sliced into smaller haplotype fragments and alleles, and haplotype/allele frequencies were calculated using parental haplotypes, assuming that all parents are unrelated individuals. In families where not all individuals were successfully typed by long range PCR or when individuals of the same family were typed by reagents from different vendors resulting in different ambiguous typing strings, automated haplotype assignment was not possible and manual haplotype assignment was performed.

Haplotypes from seven of the participating laboratories were compared for different haplotype blocks and at different typing resolutions using the local haplotype frequencies estimated as described above. Haplotype overlap between the seven groups was estimated for different haplotype groups and at different resolutions as a fraction of the total number of haplotypes in each studied group. An added haplotype overlap analysis was performed using the seven studied groups plus a group of families from Jamaica. Samples of the families from Jamaica were of inadequate quantity/quality and failed testing by long range PCR. They were typed by exon-based NGS reagents from Scisco Genetics. This additional overlap analysis was performed at 2-field resolution using 5-locus (HLA-A~HLA-C~HLA-B~HLA-DRB1~HLA-DQB1) haplotypes. Principal Component analysis were generated using Matlab R2017b (Mathworks, Inc., Natick, MA, USA). Venn diagrams were generated using the Venn Diagram tool developed by the VIB-Ugen Center for Plant Systems Biology at Ghent University (https://omictools.com/blog/your-top-3-venn-diagram-tools)

Ethical approval

The study was approved by the institutional review board (IRB) offices of Baylor Scott & White Research Institute (IRB# 017–066) and Stanford University (IRB #38899). To meet the data sharing and data storage requirements, all samples and data submitted were de-identified and did not contain personally identifiable patient information or "Protected Health Information" as defined under the United States Health Insurance Portability and Accountability Act (HIPAA), http://www.hhs.gov/hipaa/index.html.

3. Results and discussion

3.1. Alleles identified

In this study most of the analyses focused on 245 families from 7 countries (901 subjects/944 haplotypes) typed by long range PCR with 3rd/4th field resolution. The dataset also included 5 additional families from Jamaica (20 subjects/17 haplotypes) typed by exon-based typing due to sample limitations. The entire study cohort included 250 families (921 subjects/961 haplotypes). We report 11-locus haplotype associations for 486 HLA alleles including HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, -DPB1 loci (77, 115, 68, 69, 10, 6, 4, 44, 31, 20 and 42 alleles, respectively). The number of subjects tested and families included, separated by country, are presented in Table 2. Table 3 shows the populations of the study. Listings of all alleles identified, their frequencies and count of subjects carrying them in the entire cohort as well as in individual participating laboratories are shown in Supplemental Tables (S1–S11). Despite the poor quality of the specimens from Jamaica, typing was successful in all individuals and all loci (except for failed DPB1 typing on two subjects due to low DNA quantity) using exon-based amplification but not long-range PCR. Of particular interest in the presented data are the description of haplotypic association of loci that are not well published such as DQA1, DPA1, and to some extent DPB1. That also applies to alleles seen in all loci observed in families from parts of the world that are less represented in large international registries and published literature.

Due to the lack of 3rd and 4th field resolution HLA allele frequency data in the published literature, the relative frequency of the identified alleles in this study is compared to published lower resolution frequency data reported in respective countries whenever available. Results are presented in a descending order based on the number of families contributed from each country.

Table 1
Genomic regions interrogated by each kit used in the project.

Reagent (alphabetically)	A & C	B	DRB1/3/4/5	DQA1	DQB1	DPA1	DPB1
Gen Dx Products, Inc;	Full gene	Full gene	Exons 2& 3	Full gene	exons 2&3 part of 4	Exons 2&3, part of 1 and 4	Full gene (5'UTR- Intron 1 + Intron 1 – 3' UTR)
Illumina, Inc;	Full gene	Exon 1-Intron 6	Exon 2-intron 4,	Full gene	Full gene	Full gene	Exon 2-3'UTR
Immucor, Inc;	Full gene	Full gene	Exons 1 + 2–6	Full gene	Full gene	Full gene	Exons 2–4
Omixon, Inc;	Full gene	Full gene	Exons 2–5	Full gene	Full gene	Full gene	Exon 2-3'UTR
Scisco Genomics(Exon based)	Exons 1–7	Exons 1–7	Exons 1–4	Exons 1–4	Exons 1–4	Exons 1–4	Exons 1–4
Therom Fisher Scientific	Full gene	Full gene	Exon 2-3'UTR	Full gene	Exon 2-3'UTR	Full gene	Exon 2-3'UTR

Table 2
Parent, subject and haplotype count in each studied family group by country of contributing laboratory.

Group	Parent Count	Subject Count	Haplotype Count
Argentina	174	282	320
Austria	20	39	40
Egypt	28	73	56
Jamaica	9	20	17
Germany	138	242	270
Greece	50	96	100
Kuwait	58	116	115
Switzerland	22	53	43
Total	499	921	961

3.2. HLA alleles from Argentina

A recent study characterized the HLA diversity in 1472 unrelated volunteers of the unrelated donor registry in Argentina using NGS reported at 2nd, 3rd and/or 4th field resolution [34]. Interestingly, all most commonly seen HLA class I alleles with frequency > 5% in that study (HLA-A*02:01:01:01, HLA-A*01:01:01:01, HLA-A*24:02:01:01, HLA-A*31:01:02:01, HLA-A*03:01:01:01, HLA-A*11:01:01:01, HLA-B*35:01:01:01, HLA-B*51:01:01:01, HLA-B*44:03:01:01, HLA-B*07:02:01, HLA-C*04:01:01:01, HLA-C*07:01:01:01, and HLA-C*12:03:01:01) were among the top 10 alleles observed in our study. Five of the six HLA-DRB1 alleles with a frequency > 5% (HLA-DRB1*07:01:01, HLA-DRB1*03:01:01, HLA-DRB1*13:01:01, HLA-DRB1*15:01:01, HLA-DRB1*11:04:01, HLA-DRB1*01:01:01) and all six DQB1 alleles with a frequency > 5% (HLA-DQB1*03:02:01, HLA-DQB1*03:01:01, HLA-DQB1*02:02:01, HLA-DQB1*02:01:01, HLA-DQB1*06:02:01, HLA-DQB1*05:01:01:01) were among the top 10 alleles observed in our study as well. In addition, allele frequencies calculated from a healthy Argentinian cohort of 1459 samples were reported in [35] at the allele-family resolution. The eight HLA-DRB1 allele families reported in [35] with frequencies > 10% overlapped with allele families of 30 out of 45 HLA-DRB1 alleles reported in our study in Argentinian samples.

3.3. HLA alleles from Germany

A catalog of common and well documented (CWD) HLA alleles of German stem cell donors was recently reported at 2-field resolution including alleles at the six HLA loci A, B, C, DRB1, DQB1, and DPB1 [36]. HLA-A alleles (HLA-A*02:01:01:01, HLA-A*03:01:01:01, HLA-A*01:01:01:01, HLA-A*24:02:01:01, HLA-A*11:01:01:01) and HLA-DPB1 alleles (HLA-DPB1*04:01:01, HLA-DPB1*02:01, HLA-DPB1*04:02:01:02, HLA-DPB1*03:01:01, HLA-DPB1*01:01:01) that were the most frequently observed alleles in families from Germany in our study (representing 68.5% of HLA-A alleles and 78.9% of HLA-DPB1 alleles in our study) were also the top five alleles for those loci in the CWD alleles in German population report. Similarly, the four most frequently seen family HLA-B, HLA-C, HLA-DRB1, DQB1 alleles seen in families from Germany in our study were among the top five German CWD alleles for those loci (HLA-B*07:02:01, HLA-B*08:01:01:01, HLA-B*51:01:01:01, HLA-B*44:02:01:01; HLA-C*07:02:01:03, HLA-C*07:01:01:01, HLA-C*04:01:01:01, HLA-C*12:03:01:01; HLA-DRB1*15:01:01, HLA-DRB1*07:01:01, HLA-DRB1*03:01:01, HLA-DRB1*01:01:01; and HLA-DQB1*06:02:01, HLA-DQB1*03:01:01:03, HLA-DQB1*03:02:01, HLA-DQB1*02:01:01).

3.4. HLA alleles from Kuwait

The 5 most common HLA-A, HLA-B, and HLA-C alleles seen in families from Kuwait are HLA-A *02:01:01, HLA-A*01:01:01, HLA-A*24:02:01, HLA-A*11:01:01, HLA-A* 23:01:01; HLA-B*35:01:01, HLA-B*08:01:01, HLA-B*50:01:01, HLA-B*07:02:01, HLA-B*51:01:01; and HLA-C*04:01:01, HLA-C*06:02:01, HLA-C*07:02:01, HLA-C*07:01:01, HLA-C*17:01:01. Allele ranking for the top 5 most commonly observed HLA-DRB1 alleles in families from Kuwait in our study (HLA-DRB1*03:01:01, HLA-DRB1*07:01:01, HLA-DRB1*13:02:01, HLA-DRB1*11:04:01, and HLA-DRB1*15:02:01:01) corresponded to the most common low-resolution DR specificities reported in 114 healthy Kuwaiti Arabs [37]. Four of the top five ranked HLA-DQB1 alleles in the same Kuwaiti cohort (HLA-DQB1*02:01, HLA-DQB1*03:01, HLA-DQB1*03:02, and HLA-DQB1*06:01) overlap at 2-field resolution with alleles among the top 10 observed in our study (HLA-DQB1*02:01:01, HLA-DQB1*03:01:01:03, HLA-DQB1*03:02:01, and HLA-DQB1*06:01) [38].

3.5. HLA alleles from Greece

A report of 2-field resolution level HLA haplotype and allele frequencies of stem cell donors in Germany with foreign parentage included allele frequencies at four loci (HLA-A, HLA-B, HLA-C and HLA-DRB1) in 1,894 subjects with Greek parentage [39]. Among subjects in that study and ours, the seven top HLA-A alleles (HLA-A*02:01:01:01, HLA-A*01:01:01:01, HLA-A*11:01:01:01, HLA-A*26:01:01:01, HLA-A*03:01:01:01, HLA-A*24:02:01:01, and HLA-A*32:01:01), top five HLA-B alleles (HLA-B*51:01:01:01, HLA-B*35:01:01:01, HLA-B*18:01:01:02, HLA-B*35:03:01, and HLA-B*08:01:01:01), top three HLA-C alleles (HLA-C*07:01:01:01, HLA-C*04:01:01:01, and HLA-C*12:03:01:01), and top five HLA-DRB1 alleles (HLA-DRB1*11:04:01, HLA-DRB1*11:01:01:01, HLA-DRB1*16:01:01, HLA-DRB1*07:01:01, and HLA-DRB1*03:01:01) were shared at 2-field resolution.

3.6. HLA alleles from Egypt

Of the 24 HLA-A and 25 HLA-B alleles identified in Egyptians in our study, allele families of 21 and 22 alleles overlapped with 10 out of the 13 and 15 out of the 20 low-resolution (allele-family level) HLA-A and -B specificities reported in 221 healthy Egyptian subjects, respectively [40,41]. Similarly, the six most observed DRB1 alleles in families from Egypt in our study (HLA-DRB1*03:01:01, HLA-DRB1*01:02:01, HLA-DRB1*07:01:01, HLA-DRB1*13:01:01, HLA-DRB1*11:01:01:01, HLA-DRB1*04:02:01, HLA-DRB1*04:05:01, HLA-DRB1*11:04:01, HLA-DRB1*15:02:01:01, HLA-DRB1*04:03:01, HLA-DRB1*03:02:01, HLA-DRB1*15:02:01:02, HLA-DRB1*04:04:01, HLA-DRB1*13:02:01, HLA-DRB1*11:03:01) corresponded the most commonly seen DRB1 alleles in 73 healthy Egyptian blood donors [42]. In addition, 12 of the 16 HLA-DQB1 alleles identified in Egyptians in our study overlapped with eight out of the nine 2-field resolution alleles reported in 113 healthy Egyptian subjects [43].

3.7. HLA alleles from Switzerland

The Swiss population has demonstrated a heterogeneous HLA genetic makeup [44]. The four top ranking HLA-A alleles (HLA-A*02:01:01:01, HLA-A*24:02:01:01, HLA-A*03:01:01:01, HLA-A*32:01:01) in families from Switzerland in our study corresponded to those among the six top alleles observed in a combined cohort of donors from 913 Swiss hematopoietic cell transplant donor recruitment centers reported at 2-field resolution. Three of the four top HLA-C alleles (HLA-C*04:01:01:01, HLA-C*07:01:01:01, and HLA-C*06:02:01:01) were shared between our study and the Swiss study. The four top ranked HLA-DQB1 alleles (HLA-DQB1*06:02:01, HLA-DQB1*03:01:01:02, HLA-DQB1*05:01:01:02, and HLA-DQB1*02:02:01:01) were shared between our study and the overall ranking in the Swiss study. There was no notable overlap among observed frequencies of HLA-B or -DRB1 alleles in families from Switzerland in our study and the overall frequency among donors from Swiss centers nor with frequencies in any particular region of Switzerland, including Basel where the participating Swiss laboratory is located.

Table 3
Most frequently seen alleles (at loci HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1) in the entire cohort as well as their frequency ranking in 7 countries.

	Allele	Global Rank	ARG Rank	AUS Rank	EGY Rank	GER Rank	GRK Rank	KU Rank	SWI Rank
HLA-A	HLA-A*02:01:01:01	1	1	1	4	1	1	–	1
	HLA-A*01:01:01:01	2	2	2	1	3	2	2	11
	HLA-A*24:02:01:01	3	3	7	2	4	5	3	1
	HLA-A*03:01:01:01	4	5	13	13	2	5	6	3
	HLA-A*11:01:01:01	5	6	4	13	5	3	4	–
	HLA-A*26:01:01:01	6	10	3	3	6	4	13	11
	HLA-A*32:01:01	7	8	7	13	8	7	7	4
	HLA-A*23:01:01	8	10	7	8	7	9	5	4
	HLA-A*31:01:02:01	8	4	13	–	11	14	11	–
	HLA-A*30:01:01	10	6	4	13	9	14	11	11
HLA-B	HLA-B*51:01:01:01	1	3	1	–	3	1	4	1
	HLA-B*07:02:01	2	9	3	–	1	9	4	5
	HLA-B*08:01:01:01	3	1	–	6	2	4	–	–
	HLA-B*35:01:01:01	4	2	9	3	6	2	–	11
	HLA-B*38:01:01	5	9	5	3	3	20	23	5
	HLA-B*13:02:01	6	4	9	–	9	8	15	5
	HLA-B*44:02:01:01	7	6	9	–	3	12	–	5
	HLA-B*18:01:01:02	8	12	3	9	9	3	–	–
	HLA-B*35:03:01	9	16	–	14	7	4	15	11
	HLA-B*44:03:01:01	9	5	9	14	11	20	23	–
HLA-C	HLA-C*04:01:01:01	1	1	3	4	3	1	2	1
	HLA-C*07:01:01:01	2	2	4	2	2	1	5	3
	HLA-C*12:03:01:01	3	5	1	2	4	3	9	5
	HLA-C*06:02:01:01	4	4	2	9	5	10	2	3
	HLA-C*07:02:01:03	5	13	4	–	1	10	5	5
	HLA-C*04:01:01:06	6	8	11	5	8	5	1	–
	HLA-C*07:02:01:01	7	3	11	9	13	–	5	5
	HLA-C*01:02:01	8	7	11	14	8	8	14	5
	HLA-C*03:03:01:01	9	6	11	–	12	6	16	–
	HLA-C*15:02:01:01	10	8	4	–	11	8	9	15
HLA-DRB1	HLA-DRB1*03:01:01:01/HLA-DRB1*03:01:01:02	1	2	2	1	2	5	1	–
	HLA-DRB1*07:01:01:01/HLA-DRB1*07:01:01:02	2	1	5	3	2	4	2	2
	HLA-DRB1*11:04:01	4	6	1	8	7	1	4	3
	HLA-DRB1*15:01:01:01/HLA-DRB1*15:01:01:02/HLA-DRB1*15:01:01:03	3	6	11	23	1	6	–	3
	HLA-DRB1*11:01:01:01	5	4	2	5	6	2	6	5
	HLA-DRB1*13:01:01:01/HLA-DRB1*13:01:01:02	6	3	5	3	4	9	6	10
	HLA-DRB1*01:01:01	7	11	5	–	5	12	9	5
	HLA-DRB1*13:02:01	8	10	11	13	8	16	3	7
	HLA-DRB1*16:01:01	9	13	5	21	9	3	17	–
	HLA-DRB1*04:04:01	10	8	11	13	12	–	17	–
HLA-DQB1	HLA-DQB1*03:02:01	1	1	6	3	4	3	7	7
	HLA-DQB1*03:01:01:03	2	8	1	10	2	5	2	11
	HLA-DQB1*02:01:01	4	4	3	1	4	6	1	–
	HLA-DQB1*06:02:01	3	9	3	–	1	6	10	1
	HLA-DQB1*02:02:01:01	5	3	3	2	7	3	3	4
	HLA-DQB1*06:03:01	6	7	6	5	3	12	7	11
	HLA-DQB1*03:01:01:02	7	6	12	7	15	1	17	2
	HLA-DQB1*03:01:01:01	8	2	6	8	9	10	17	11
	HLA-DQB1*04:02:01	9	5	12	8	12	–	5	4
	HLA-DQB1*05:02:01	10	11	2	12	8	2	7	4
HLA-DPB1	HLA-DPB1*04:01:01:01/HLA-DPB1*04:01:01:02	1	1	1	1	1	1	1	1
	HLA-DPB1*02:01:02/HLA-DPB1*02:01:19	2	3	2	2	2	2	2	2
	HLA-DPB1*04:02:01:02	3	2	3	3	3	6	5	3
	HLA-DPB1*03:01:01	4	4	6	9	4	4	10	7
	HLA-DPB1*104:01	5	11	3	7	6	8	3	5
	HLA-DPB1*01:01:01	6	6	7	4	5		6	6
	HLA-DPB1*04:02:01:01	7	7		9	8	3	12	5
	HLA-DPB1*14:01:01	7	8	3	9	9	7	3	7
	HLA-DPB1*13:01:01/HLA-DPB1*107:01	9	8		4	11	9	6	5
	HLA-DPB1*17:01	10	15	7	4	6	10	6	7

3.8. HLA alleles from Austria and Jamaica

The combination of the relatively small number of subjects from Austria and Jamaica included in our study and the lack of published reports on HLA allele frequencies in these two countries precluded meaningful comparison of our results to existing literature. Since individuals from families from Jamaica were only successfully tested by exon-based sequencing due to sample quality, only 3rd field resolution of alleles could be determined. Table S12a presents all alleles identified in families from Jamaica.

3.9. Novel variants

Most families tested had one or more variant sequences in non-coding regions at least at one locus. Nine novel alleles with

polymorphisms in coding regions were identified in our study. All novel alleles identified using one NGS platform were confirmed using another platform. These alleles included A*26:variant, B*variant (B*18:01xB*27:07), DRB3*01:variant (seen on two distinct haplotypes from two unrelated families from Jamaica), DRB3*02:variant, DQB1*03:variant, DQB1*02:89, DPA1*01:14, DPA1*03:01:02, DPB1*16:variant. DQB1*02:89, DPA1*01:14, DPA1*03:01:02 have been submitted to IMGT/HLA database while submission of the remaining alleles is in progress [45]. Details of the genetic polymorphism, amino acid substitution (if applicable), associated haplotype, and country in which these novel alleles appeared are presented in Supplemental Table S13. Submission of novel alleles that have not been assigned official names to Gene Bank & IMGT/HLA databases is in progress.

3.10. Haplotypes identified

The number of haplotypes identified, separated by country, are presented in Table 2. The existence of a hot spot of recombination between DP loci and DQ loci has been reported previously [8,9,46]. We have identified recombination events between DP and DQ loci in 2 of the 128 quartet families we tested, a finding that is discussed later. In addition, we observed that many identical haplotypes across nine loci become divergent in terms of the multiplicity of DP alleles with which they associate. The 2nd and 3rd 11-locus haplotypes most commonly identified among all tested individuals were identical across nine loci and differ only in DPA1/DPB1 alleles. Haplotype information, including frequency, family count, and subject count are listed in Supplemental Table S14 for the seven main studied groups for a total of 944 11-locus HLA-A~HLA-C~HLA-B~HLA-DRB3/4/5~HLA-DRB1~HLA-DQA1~HLA-DQB1~HLA-DPA1~HLA-DPB1 haplotypes, all at 3rd - or 4th -field resolution. The 9-locus haplotype information (excluding DPA1 & DPB1), including frequency, family count and subject count is listed in Supplemental Table S15. To test whether this conversion of haplotypes was the function of excluding the most distant loci of the haplotype, we grouped 10-locus haplotypes excluding A locus only and didn't observe similar effect. The 3 top ranking 10-locus haplotypes (excluding A locus and including DP loci, Supplemental Table S16) and 9-locus haplotypes (including A locus and excluding DP loci) were seen in 9, 8, 7 and 28, 12, 7 families, respectively.

Due to the paucity of literature describing 4th field level resolution haplotypes and particularly in non-Caucasian populations, haplotypes were further grouped based on 2-field resolution to conduct comparisons to published haplotype frequencies. Supplemental Table S17 lists 2-field resolution 5-locus haplotypes and their frequency in studied populations as well as their frequency and ranking in major US populations [16]. The top ranking 10 of these haplotypes are presented in Table 4.

3.11. Overlap of haplotypes among families from different countries

We attempted to visualize haplotype overlap and genetic proximity of the studied populations using multiple methods. Supplementary Fig. S1 shows the result of a principal component analysis (PCA) conducted using the estimated haplotype frequencies. We repeated the PCA using 2-field HLA resolution which led to collapsing some of the original haplotypes resulting in a total of 736 haplotypes at the 2-field resolution (Supplemental Fig. S2). While the PCA plot shows clustering of families from Argentina, Germany, Greece and Kuwait, it doesn't necessarily imply genetic proximity of the clustered populations. Supplementary Fig. S3 depicts a Venn diagram of the haplotype overlap among these four populations. There was no significant haplotype overlap between these five populations.

Due to the relatively small size of some of the studied groups, there was sparse haplotype overlap most of the time. Argentina, Germany, and Kuwait were the groups with the largest sample sizes (Table 2) and therefore shared some haplotypes with most groups, while Switzerland, Austria, and Egypt had very few haplotypes in common with other groups at the studied resolution (Figs. S1 and S2). The number of families from Jamaica was the smallest of all studied groups with only 17 haplotypes in total and only one haplotype at the 5-locus 2-field resolution (HLA-A*01:01~HLA-C*07:01~HLA-B*08:01~HLA-DRB1*03:01~HLA-DQB1*02:01) shared with all groups except Austria, Egypt, and Switzerland. Table S12b shows all haplotypes identified in the families from Jamaica. This shared haplotype is also the most common haplotype in most European populations and US European [16]. One might expect some overlap in haplotypes among families from Germany and those from its neighboring German speaking countries (Austria and Switzerland). The fact that we did not observe such overlap might be a function of the relatively small number of families studied from each country, particularly Austria and Switzerland. It could also reflect some level of heterogeneity among these populations [39,44].

3.12. Partial haplotypes

Most studied groups share at least one haplotype at the 2-field 5-locus (HLA-A~HLA-C~HLA-B~HLA-DRB1~HLA-DQB1) resolution. While most haplotypes were private to each group, some 2- and 3-locus blocks were common across the board. The top 2-locus blocks shared by all seven groups were:

HLA-C*12:03:01:01~HLA-B*38:01:01 /
HLA-C*07:01:01:01~HLA-B*49:01:01
HLA-DRB3*02:02:01:02~HLA-DRB1*11:04:01 /
HLA-DRB3*02:02:01:02~HLA-DRB1*11:01:01:01
HLA-DRB1*07:01:01:01/HLA-DRB1*07:01:01:02~HLA-DQB1*02:02:01:01 /
HLA-DRB1*13:01:01:01/HLA-DRB1*13:01:01:02~HLA-DQB1*06:03:01
HLA-DQA1*03:01:01~HLA-DQB1*03:02:01 /
HLA-DQA1*02:01:01:01/HLA-DQA1*02:01:01:02~HLA-DQB1*02:02:01:01
HLA-DPA1*01:03:01:04~HLA-DPB1*04:01:01:01/HLA-DPB1*04:01:01:02 /
HLA-DPA1*01:03:01:02~HLA-DPB1*04:01:01:01/HLA-DPB1*04:01:01:02.

Supplemental Table S12b shows all 17 haplotypes identified in families from Jamaica. Considering haplotype blocks > 2-locus, here are the top shared haplotypes among the seven studied groups:

- HLA-A*01:01:01:01~HLA-C*07:01:01:01~HLA-B*49:01:01 shared by all except Switzerland
- HLA-C*12:03:01:01~HLA-B*38:01:01~HLA-DRB1*13:01:01:01/HLA-DRB1*13:01:01:02~HLA-DQB1*06:03:01 shared by all except Greece and Kuwait
- HLA-DRB4*01:03:01:01/HLA-DRB4*01:03:01:03~HLA-DRB1*07:01:01:01/HLA-DRB1*07:01:01:02~HLA-DQA1*02:01:01:01/HLA-DQA1*02:01:01:02~HLA-DQB1*02:02:01:01
- HLA-DRB1*01:02:01~HLA-DQA1*01:01:02~HLA-DQB1*05:01:01:01

3.13. Recombinant haplotypes

We identified 4 recombinant haplotypes in the 128 quartet families included in the study. Two haplotypes showed a crossover between class I and class II loci and the other 2 showed a crossover between DQ and DP loci. Crossover couldn't be determined reliably among trio families. Supplemental Tables S27–S30 show segregation in families carrying the recombinant haplotypes.

Table 4
The top ranking 2-field resolution 5-locus haplotypes and their frequency ranking in studied countries and major US populations.

Haplotype	Global	Argentina	Austria	Egypt	Germany	Greece	Kuwait	Switzerland	HIS_Rank	SCAMB_Rank	AMIND_Rank
A*01:01~C*07:01~B*08:01~DRB1*03:01~DQB1*02:01	1	1	0	0	1	1	2	0	1	1	1
A*03:01~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	2	6	0	0	2	3	0	0	3	3	2
A*02:01~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	3	6	2	0	3	0	3	0	8	10	4
A*26:01~C*12:03~B*38:01~DRB1*13:01~DQB1*06:03	4	3	2	0	7	0	0	0	49	104	189
A*30:01~C*06:02~B*13:02~DRB1*07:01~DQB1*02:01	5	2	2	0	0	0	0	0	13	45	23
A*24:02~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	6	5	0	0	4	0	0	0	22	36	13
A*24:02~C*04:01~B*35:01~DRB1*04:02~DQB1*03:02	7	0	0	2	0	3	2	0	695		932
A*23:01~C*04:01~B*44:03~DRB1*07:01~DQB1*02:01	8	6	0	3	5	0	0	0	14	43	22
A*02:01~C*12:02~B*52:01~DRB1*15:02~DQB1*06:01	9	6	0	0	7	3	3	0	51		486
A*02:01~C*06:02~B*13:02~DRB1*07:01~DQB1*02:01	10	0	0	0	5	0	3	0	45	298	30

Haplotype	AISC_Rank	CARHIS_Rank	FILII_Rank	SCAHIS_Rank	AFA_Rank	HAWI_Rank	CAU_Rank	NAMER_-Rank	JAPI_Rank	VIET_Rank	CARB_Rank	MSWHIS_Rank
A*01:01~C*07:01~B*08:01~DRB1*03:01~DQB1*02:01	1	2	48	2	2	9	1	1	51	221	5	1
A*03:01~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	2	4	95	3	6	18	2	2	131	262	11	3
A*02:01~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	3	16	181	7	14	45	3	4	212	603	22	10
A*26:01~C*12:03~B*38:01~DRB1*13:01~DQB1*06:03	65	104	1055	40	503	488	47	58	2458	2176	727	59
A*30:01~C*06:02~B*13:02~DRB1*07:01~DQB1*02:01	20	53	42	13	71	162	11	12	114	24	92	14
A*24:02~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	12	40	286	16	50	135	12	11	138	924	98	31
A*24:02~C*04:01~B*35:01~DRB1*04:02~DQB1*03:02	5392	987	1357	857	6414	931	576	742			10658	577
A*23:01~C*04:01~B*44:03~DRB1*07:01~DQB1*02:01	16	27	434	11	42	82	13	14	914	5992	32	21
A*02:01~C*12:02~B*52:01~DRB1*15:02~DQB1*06:01	139	47	980	54	1532	798	95	141	72	2204	1372	77
A*02:01~C*06:02~B*13:02~DRB1*07:01~DQB1*02:01	22	141	451	39	197	245	15	16	431	1010	334	44

Haplotype	AFB_Rank	SCSEAI_R-ank	ALANAM_-Rank	AAFA_Rank	NAM_Rank	AINDI_Ran-k	CARIBI_Ra-nk	API_Rank	NCHI_Rank	KORI_Rank	MENAF-C_Rank
A*01:01~C*07:01~B*08:01~DRB1*03:01~DQB1*02:01	4	131	1	2	1	1788	3	100	466	154	1
A*03:01~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	9	64	5	5	2	75	4	61	121	212	5
A*02:01~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	31	152	10	13	4	288	28	209	438	415	10
A*26:01~C*12:03~B*38:01~DRB1*13:01~DQB1*06:03	446	246	207	504	129	258	157	513	3235	919	21
A*30:01~C*06:02~B*13:02~DRB1*07:01~DQB1*02:01	132	6	35	72	21	6	124	6	4	5	4
A*24:02~C*07:02~B*07:02~DRB1*15:01~DQB1*06:02	174	30	36	49	14	29	85	51	177	266	23
A*24:02~C*04:01~B*35:01~DRB1*04:02~DQB1*03:02	4577			6495	1095	5524	3239	6162	18639		79
A*23:01~C*04:01~B*44:03~DRB1*07:01~DQB1*02:01	42	180	46	42	19	154	62	312	869	3015	16
A*02:01~C*12:02~B*52:01~DRB1*15:02~DQB1*06:01	876	52		1923	317	38	27	65	421	232	40
A*02:01~C*06:02~B*13:02~DRB1*07:01~DQB1*02:01	257	243	42	183	28	435	303	216	148	237	22

3.14. Study design considerations and limitations

It is worth noting that the haplotype frequencies reported in this article were calculated from HLA collected predominantly from patient families and therefore not necessarily representative of their respective populations at large. While the reported frequencies can be used as an indication of some of the common HLA alleles and haplotypes in certain communities, this study was not designed as an anthropological analysis. Neither the ethnic nor racial background of any study subject was ascertained. Rather this is a collection of extended haplotypes of subjects living in the countries where participating laboratories exist. Therefore, the reported frequency values should be interpreted with caution and may not mimic values calculated from larger samples collected from unrelated individuals. It is not unconceivable that biases in patient and patient family HLA may exist with possible associations with certain disease traits. Other non-genetic influences include selection bias due to disease diagnoses and access to healthcare. These factors can result in family allele and haplotype frequencies not reflective of the larger population [47,48]. Considering the non-random nature of the subjects studied, we cannot make generalizable inferences about the frequencies of alleles or haplotypes observed. However, we present the haplotype associations observed in this study that could be validated in future studies designed specifically to estimate allele and haplotype frequencies in these studied and closely related populations. In addition, in this study we report 11-locus haplotype associations at allele-level resolution (3rd field and when possible/applicable 4th field) of 486 alleles identified by segregation in families from eight countries and four continents. Comparison with published literature identified overlap between the alleles we report (at lower resolutions) and the most frequently observed lower resolution results reported in the studied populations. This report also describes a model for collaboration under the 17th IHIW in which a core laboratory using an emerging technology could perform testing for numerous laboratories at various stages of implementation of the technology and facilitate exchange of expertise, best practices, and cross-platform validation. Although not all participating laboratories performed typing in parallel with the core laboratory, they received the typing results from the core laboratory, which often served as reference material for subsequent validation. Having core laboratories for the workshop allowed typing in a high throughput fashion and at a significantly reduced cost. As has been the tradition for international workshops, NGS vendors generously provided reagents. This core laboratory model ensured that vendors received the greatest amount of typing data as well as feedback on the performance of their platforms in a high throughput environment.

4. Future directions

A continuation of this project is open for participation under the 18th IHIW that will be held in May 2021 in Amsterdam, The Netherlands (https://www.ihiw18.org/component-ngs-for-hla/project-haplotypes-in-families/). Investigators interested in joining the project are encouraged to register through 18th IHIW web site.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.07.298.

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