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Original Article

Using high-resolution human leukocyte antigen typing of 11,423 randomized unrelated individuals to determine allelic varieties, deduce probable human leukocyte antigen haplotypes, and observe linkage disequilibria between human leukocyte antigen-B and-C and human leukocyte antigen-DRB1 and-DQB1 alleles in the Taiwanese Chinese population

Kuo-Liang Yang[a,b], Hsee-Bin Chen[a]*

[a]Laboratory of Immunogenetics, Tzu Chi Cord Blood Bank and Buddhist Tzu Chi Marrow Donor Registry, Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, Hualien, Taiwan, [b]Department of Laboratory Medicine, Buddhist Tzu Chi University, Hualien, Taiwan

Abstract

Objective: We report here the human leukocyte antigen (HLA) allelic variety and haplotype composition in a cohort of the Taiwanese Chinese population and their patterns of linkage disequilibria on HLA-B: HLA-C alleles and HLA-DRB1: HLA-DQB1 alleles at a high-resolution level. **Materials and Methods:** Peripheral whole blood from 11,423 Taiwanese Chinese unrelated individuals was collected in acid citrate dextrose. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit. The DNA material was subjected to HLA genotyping for HLA-A,-B,-C,-DRB1, and-DQB1 loci using a commercial polymerase chain reaction-sequence-based typing (PCR-SBT) kit, the SeCore® A/B/C/DRB1/DQB1 Locus Sequencing kit. High-resolution allelic sequencing was performed as previously described. **Results:** The number of individual HLA-B alleles detected was greater than the number of alleles recognized in the both the HLA-A and-DRB1 loci. Several novel alleles were discovered as a result of employing the SBT method and the high number of donors tested. In addition, we observed a genetic polymorphic feature of association between HLA-A and-B, HLA-B and-C, and HLA-DRB1 and-DQB1 alleles. Further, the homozygous haplotype frequencies of HLA-A and-B; HLA-A,-C, and-B; HLA-A,-C,-B, and-DRB1; and HLA-A,-C,-B,-DRB1, and-DQB1 in Taiwanese Chinese population are presented. **Conclusion:** As increasing number of HLA alleles are being discovered, periodic HLA profile investigation in a given population is essential to recognize the HLA complexity in that population. Population study can also provide an up-to-date strategic plan for future needs in terms of compatibility measurement for HLA matching between transplant donors and patients.

Keywords: *Alleles, Haplotypes, Human leukocyte antigen, Induced pluripotent stem cells, Linkage disequilibria, Taiwanese*

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Introduction

Transplantation of allogeneic hematopoietic stem cells has been employed as a curative therapy for hematologic malignancies and other hematologic or immune disorders. Human leukocyte antigen (HLA) molecules have been defined as transplant antigens and have a strong relevance to tissue transplantation. The molecular similarity between transplant donors and recipients is considered a predictive factor for graft survival and graft versus host disease as it can elicit immune responses by either recognition of polymorphic fragments of foreign HLA molecules or presentation of various peptides [1-3]. The genes encoding the HLA alleles are located in the Major Histocompatibility Complex Class I and II regions. The HLA genes are characterized by their extreme allelic polymorphism as well as their variations and diversity in different ethnic groups. The number of known HLA alleles is increasing dramatically with the development of DNA-based molecular typing technology [4]. Understanding the HLA diversity in ethnic groups is

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*Address for correspondence:
Prof. Kuo-Liang Yang,
Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan.
E-mail: edward@tzuchi.com.tw

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important. Facilitating appropriate HLA-matched unrelated bone marrow stem cell donors for successful stem cell transplantations relies on the accuracy of HLA typing and the ability to resolve problems with unknown, ambiguous, and low-incidence genes in the HLA system. In addition, determination of HLA haplotypes is essential for matching between donor and recipient in unrelated stem cell transplantation since it increases the likelihood of matching at other loci within the HLA region compared with matching only at the individual allelic level [5].

Determination of HLA haplotypes can be done by HLA typing of blood-related family members and prediction from large-sized population tissue typing [5-7]. Alternatively, it can be achieved by deducing typing results from donors with allelic homozygosities in the HLA-A,-B, and-DRB1 loci [8]. In family study, segregation of HLA individual alleles provides evidence of allelic linkages [9]. In population study, determination of haplotypes involves noting whether alleles at other loci are consistently present and family study is not performed. Instead, most available haplotype data are derived from studies of unrelated individuals in whom the putative haplotype is defined by statistical association analysis [6]. Linkage disequilibrium (LD), a phenomenon whereby certain combinations of alleles occur in HLA haplotypes within a population more frequently than expected based on gene frequencies, is commonly observed and has important clinical and biological implications [9]. The alleles of HLA-B and-C as well as HLA-DRB1 and-DQB1 are known to have strong LD, yet very few LD reports on the high-resolution allelic level are available in the Taiwanese Chinese population.

We report here the HLA allelic variety and haplotype composition of a cohort of the Taiwanese Chinese population consisting 11,423 unrelated individuals and show the patterns of LD on HLA-B: HLA-C alleles and HLA-DRB1: HLA-DQB1 alleles at the high-resolution level found in Taiwanese Chinese. We believe that as increasing number of HLA alleles are being discovered, periodic HLA profile investigation in a given population is essential to recognize the HLA complexity in that population. Population study can also provide an up-to-date strategic plan for future needs in terms of compatibility measurement for HLA matching between transplant donors and patients.

Materials and methods

Human leukocyte antigen DNA typing

The population of Taiwanese Chinese includes four ethnic groups (those whose ancestors migrated from Fujian province and Guangdong province, mainland Chinese from provinces other than Fujian and Guangdong, and native aborigines). Peripheral whole blood samples of Taiwanese Chinese were collected in acid citrate dextrose (ACD) anticoagulant from Taiwanese Chinese unrelated volunteer bone marrow donors with formal written consent at routine blood drives.

Aliquots of the ACD whole blood were stored frozen at −80°C until use. Peripheral blood genomic DNA was extracted using the QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The DNA material was subjected to HLA genotyping for HLA-A,-B,-C,-DRB1, and-DQB1 loci using a commercial polymerase chain reaction- sequence-based typing (PCR-SBT) kit, the SeCore® A/B/C/DRB1/DQB1 Locus Sequencing kit (Life Technologies, Brown Deer, WI, USA). High-resolution allelic sequencing was performed as previously described [10,11].

Determination of linkage disequilibrium by statistical analysis

The frequencies of HLA Class I (HLA-A,-B, and-C) and II (HLA-DRB1 and-DQB1) alleles were calculated by direct counting, and the patterns of extended haplotypes were estimated using the maximum-likelihood method with the expectation–maximization (EM) algorithm using Arlequin version 3.5 [12].

Results

Human leukocyte antigen-A,-B,-C,-DRB1, and-DQB1 alleles

Table 1 shows the number of individuals tested for each HLA-A, -B,-C,-DRB1, and-DQB1 loci and the number of distinctive alleles identified in each locus in this study. The frequencies of the alleles identified are listed in Appendix 1. Almost an equal number of donors were tested for HLA-A,-B, and-DRB1 loci, and we found that the number of the alleles detected in the HLA-B locus exceeded the number of alleles found in both the HLA-A and-DRB1 loci. In Table 2, the homozygous haplotype frequencies of HLA-A and-B; HLA-A,-C, and-B; HLA-A,-C,-B, and-DRB1; and HLA-A,-C,-B,-DRB1, and-DQB1 in the Taiwanese Chinese population are presented. In general, the frequency of homozygous haplotypes decreased as the number of loci increased.

In the HLA-A locus, we found that the alleles with a frequency higher than 1% were A*11:01 (28.36%), A*24:02 (17.29%), A*02:07 (11.76%), A*02:01 (10.62%), A*33:03 (9.78%), A*02:03 (6.49%), A*11:02 (4.07%), A*02:06 (2.82%), A*26:01 (2.22%), A*30:01 (1.96%), and A*31:01 (1.78%) [Appendix 1].

For the HLA-B locus, the alleles detected with a frequency above 1% were B*40:01 (21.04%), B*46:01 (14.03%), B*58:01 (9.60%), B*13:01 (6.51%), B*15:02 (5.43%), B*51:01 (4.46%), B*38:02 (4.17%), B*15:01 (4.03%), B*54:01 (3.23%), B*55:02

Table 1: Number of Taiwanese Chinese tested and number of alleles identified

HLA locus	A	B	C	DRB1	DQB1
Number of individuals tested	11364	11364	5472	11354	3190
Number of alleles identified	52	100	56	79	41

HLA: Human leukocyte antigen

Table 2: Homozygosity of Taiwanese Chinese

HLA haplotype	A-B	A-B-C	A-B-DRB1	A-B-C-DRB1	A-B-C-DRB1-DQB1
Number of individuals tested	11,356	5419	11,340	5409	2947
Number of individuals with homozygosity (%)	358 (3.15)	110 (2.03)	114 (1.01)	43 (0.80)	14 (0.48)

HLA: Human leukocyte antigen

Table 3: Association of human leukocyte antigen-B alleles with the three most commonly observed human leukocyte antigen-A alleles in Taiwanese Chinese

A-B haplotype	Frequency	A-B haplotype	Frequency	A-B haplotype	Frequency
02:07-07:02	0.00019	11:01-18:01	0.00045	24:02-15:03	0.00005
02:07-08:01	0.00008	11:01-18:02	0.00071	24:02-15:05	0.00004
02:07-13:01	0.00119	11:01-27:04	0.00499	24:02-15:07	0.00037
02:07-13:02	0.00019	11:01-27:05	0.00013	24:02-15:09	0.00004
02:07-15:01	0.00158	11:01-27:06	0.00029	24:02-15:11	0.00049
02:07-15:02	0.00230	11:01-35:01	0.00618	24:02-15:12	0.00020
02:07-15:11	0.00029	11:01-35:03	0.00015	24:02-15:13	0.00004
02:07-15:18	0.00031	11:01-35:05	0.00030	24:02-15:18	0.00086
02:07-15:25	0.00018	11:01-35:42	0.00004	24:02-15:25	0.00231
02:07-18:02	0.00002	11:01-37:01	0.00014	24:02-15:27	0.00186
02:07-27:04	0.00217	11:01-38:01	0.00009	24:02-18:01	0.00009
02:07-27:06	0.00001	11:01-38:02	0.00424	24:02-18:02	0.00007
02:07-27:07	0.00002	11:01-38:15	0.00004	24:02-27:04	0.00241
02:07-35:01	0.00032	11:01-39:01	0.00820	24:02-27:06	0.00023
02:07-35:05	0.00017	11:01-39:02	0.00004	24:02-27:07	0.00029
02:07-35:11	0.00004	11:01-39:05	0.00038	24:02-35:01	0.00706
02:07-37:01	0.00005	11:01-39:09	0.00005	24:02-35:02	0.00049
02:07-38:02	0.00076	11:01-39:15	0.00010	24:02-35:05	0.00080
02:07-39:01	0.00070	11:01-40:01	0.07875	24:02-37:01	0.00049
02:07-39:05	0.00005	11:01-40:02	0.00332	24:02-38:02	0.00333
02:07-40:01	0.00686	11:01-40:03	0.00006	24:02-39:01	0.00585
02:07-40:02	0.00054	11:01-40:06	0.00127	24:02-39:05	0.00063
02:07-40:06	0.00065	11:01-40:40	0.00006	24:02-40:01	0.05103
02:07-40:55	0.00005	11:01-44:03	0.00051	24:02-40:02	0.00661
02:07-46:01	0.09064	11:01-46:01	0.01898	24:02-40:03	0.00011
02:07-47:01	0.00004	11:01-46:13	0.00004	24:02-40:06	0.00718
02:07-48:01	0.00078	11:01-48:01	0.00114	24:02-40:11	0.00005
02:07-51:01	0.00253	11:01-48:03	0.00016	24:02-40:247	0.00004
02:07-51:02	0.00026	11:01-51:01	0.01312	24:02-40:76	0.00004
02:07-52:01	0.00019	11:01-51:02	0.00513	24:02-44:02	0.00077
02:07-54:01	0.00101	11:01-51:06	0.00013	24:02-46:01	0.00903
02:07-55:01	0.00009	11:01-51:07	0.00013	24:02-48:01	0.00607
02:07-55:02	0.00055	11:01-52:01	0.00177	24:02-48:03	0.00062
02:07-55:04	0.00007	11:01-54:01	0.01028	24:02-50:01	0.00004
02:07-56:01	0.00010	11:01-55:01	0.00018	24:02-51:01	0.01056
02:07-56:04	0.00003	11:01-55:02	0.01283	24:02-51:02	0.00256
02:07-58:01	0.00216	11:01-55:04	0.00064	24:02-51:07	0.00004
02:07-67:01	0.00051	11:01-55:12	0.00079	24:02-52:01	0.00102
11:01-07:02	0.00044	11:01-56:01	0.00085	24:02-54:01	0.01381
11:01-07:05	0.00006	11:01-56:03	0.00212	24:02-55:01	0.00004
11:01-08:01	0.00016	11:01-56:04	0.00017	24:02-55:02	0.00517
11:01-13:01	0.03660	11:01-57:01	0.00009	24:02-55:04	0.00009
11:01-13:02	0.00215	11:01-58:01	0.00390	24:02-55:12	0.00004
11:01-15:01	0.01842	11:01-81:01	0.00005	24:02-56:01	0.00151
11:01-15:02	0.03606	11:01-81:02	0.00049	24:02-56:03	0.00012
11:01-15:11	0.00101	24:02-07:02	0.00074	24:02-56:04	0.00031
11:01-15:12	0.00026	24:02-07:06	0.00031	24:02-57:01	0.00023
11:01-15:17	0.00004	24:02-08:01	0.00020	24:02-58:01	0.00302
11:01-15:18	0.00080	24:02-13:01	0.01150	24:02-58:03	0.00004
11:01-15:25	0.00262	24:02-13:02	0.00036	24:02-67:01	0.00054
11:01-15:27	0.00133	24:02-15:01	0.00488	24:02-81:02	0.00035
11:01-15:58	0.00037	24:02-15:02	0.00596		

(2.87%), B*27:04 (2.78%), B*39:01 (2.49%), B*35:01 (2.36%), B*13:02 (2.24%), B*40:02 (1.70%), B*40:06 (1.52%), B*48:01 (1.31%), and B*51:02 (1.23%) [Appendix 1].

The alleles we found with a frequency exceeding 1% in the HLA-C locus were C*07:02 (20.91%), C*01:02 (19.03%), C*03:04 (12.49%), C*08:01 (8.93%), C*03:02 (8.55%), C*03:03 (5.55%),

Table 4: Association of human leukocyte antigen-C alleles with the three most commonly observed human leukocyte antigen-B alleles in Taiwanese Chinese

B-C haplotype	Frequency	B-C haplotype	Frequency	B-C haplotype	Frequency
40:01-01:02	0.00144	40:01-07:486	0.00009	46:01-08:01	0.00112
40:01-02:02	0.00009	40:01-07:56	0.00019	46:01-09:01	0.00037
40:01-02:04	0.00009	40:01-07:66	0.00037	46:01-12:02	0.00039
40:01-03:02	0.00038	40:01-08:01	0.00034	46:01-14:02	0.00009
40:01-03:03	0.00376	40:01-12:02	0.00053	46:01-15:02	0.00015
40:01-03:04	0.05142	40:01-12:03	0.00143	58:01-01:02	0.00028
40:01-03:17	0.00019	40:01-14:05	0.00009	58:01-03:01	0.00009
40:01-04:01	0.00786	40:01-15:01	0.00009	58:01-03:02	0.08315
40:01-04:03	0.00730	40:01-15:02	0.02005	58:01-03:03	0.00013
40:01-04:05	0.00019	46:01-01:02	0.12518	58:01-03:04	0.00009
40:01-04:06	0.00019	46:01-01:03	0.00410	58:01-03:36	0.00028
40:01-04:07	0.00009	46:01-01:08	0.00128	58:01-04:03	0.00009
40:01-04:82	0.00066	46:01-03:02	0.00019	58:01-07:02	0.00061
40:01-06:02	0.00009	46:01-03:03	0.00016	58:01-07:04	0.00009
40:01-07:01	0.00009	46:01-03:04	0.00083	58:01-07:06	0.00009
40:01-07:02	0.11696	46:01-04:03	0.00006	58:01-08:01	0.00009
40:01-07:27	0.00009	46:01-07:02	0.00049	58:01-12:02	0.00009

Table 5: Association of human leukocyte antigen-DQB1 alleles with the three most commonly observed human leukocyte antigen-DRB1 alleles in Taiwanese Chinese

DRB1-DQB1 haplotype	Frequency	DRB1-DQB1 haplotype	Frequency	DRB1-DQB1 haplotype	Frequency
09:01-03:01	0.00112	12:02-03:01	0.09810	15:01-05:02	0.01255
09:01-03:02	0.00047	12:02-03:03	0.00029	15:01-05:03	0.00095
09:01-03:03	0.16863	12:02-05:02	0.00530	15:01-06:01	0.04222
09:01-03:26	0.00016	12:02-05:03	0.00039	15:01-06:02	0.04423
09:01-04:01	0.00032	12:02-12:01	0.00016	15:01-06:10	0.00365
09:01-05:02	0.00015	12:02-12:02	0.00017	15:01-15:01	0.00016
09:01-05:03	0.00032	15:01-03:01	0.00063	15:01-15:02	0.00016
09:01-09:01	0.00032	15:01-03:03	0.00016	15:01-16:01	0.00016
09:01-16:02	0.00016	15:01-04:02	0.00032	15:01-16:02	0.00032
12:02-02:02	0.00016	15:01-05:01	0.00016		

Table 6: The 20 most commonly observed human leukocyte antigen-A,-B, and-C haplotypes in Taiwanese Chinese

A-B-C haplotype	Frequency	A-B-C haplotype	Frequency
02:07-46:01-01:02	0.08099	02:01-40:01-07:02	0.01587
33:03-58:01-03:02	0.06951	11:01-40:01-03:04	0.01459
11:01-40:01-07:02	0.05889	11:01-51:01-14:02	0.01424
11:01-15:02-08:01	0.03445	02:01-40:01-15:02	0.01348
11:01-13:01-03:04	0.03351	24:02-54:01-01:02	0.01228
02:03-38:02-07:02	0.02842	11:02-27:04-12:02	0.01207
30:01-13:02-06:02	0.02198	11:01-15:01-04:01	0.01069
24:02-40:01-07:02	0.02124	24:02-13:01-03:04	0.01043
24:02-40:01-03:04	0.02071	24:02-51:01-14:02	0.00942
11:01-46:01-01:02	0.01757	24:02-46:01-01:02	0.00840

C*15:02 (4.32%), C*04:01 (4.18%), C*14:02 (3.85%), C*12:02 (3.47%), C*06:02 (3.12%), and C*04:03 (1.64%) [Appendix 1].

For the HLA-DRB1 locus, the alleles observed with a frequency more than 1% were DRB1*09:01 (15.70%), DRB1*12:02 (10.39%), DRB1*15:01 (9.53%), DRB1*08:03 (8.56%), DRB1*11:01 (7.71%), DRB1*04:05 (7.52%), DRB1*03:01 (6.82%), DRB1*16:02 (5.20%), DRB1*14:54 (4.09%), DRB1*12:01 (3.38%), DRB1*04:03 (3.02%), DRB1*04:06 (2.91%), DRB1*07:01 (2.84%), DRB1*13:02 (2.34%), DRB1*15:02 (2.19%), DRB1*14:05 (2.09%), and DRB1*04:04 (1.10%) [Appendix 1].

In the HLA-DQB1 locus, the alleles identified with a frequency greater than 1% were DQB1*03:01 (20.90%), DQB1*03:03 (17.31%), DQB1*06:01 (13.30%), DQB1*05:02 (10.52%), DQB1*03:02 (6.59%), DQB1*04:01 (5.99%), DQB1*02:01 (5.61%), DQB1*06:02 (4.72%), DQB1*05:03

(4.34%), DQB1*02:02 (3.28%), DQB1*05:01 (2.71%), and DQB1*06:09 (2.27%) [Appendix 1].

Association of human leukocyte antigen-A and-B alleles

The EM algorithm predictions of linkage disequilibria in HLA-A and-B alleles in Taiwanese Chinese are shown in Appendix 2. Many HLA-A alleles were found to associate with several different types of HLA-B alleles. Table 3 shows the variable HLA-B alleles in association with the three most commonly observed HLA-A alleles (A*02:07, A*11:01, and A*24:02) in Taiwanese Chinese.

Association of human leukocyte antigen-B and-C alleles

The EM algorithm predictions of linkage disequilibria in HLA-B and-C alleles are presented in Appendix 2. Similar to the findings in the HLA-A locus, many HLA-B alleles were observed to link heterogeneously with several types of HLA-C alleles. Table 4 illustrates the variable HLA-C alleles in association with the three most frequently detected HLA-B alleles (B*40:01, B*46:01, and B*58:01) in Taiwanese Chinese.

Association of human leukocyte antigen-DRB1 and-DQB1 alleles

The EM algorithm predictions of linkage disequilibria in the HLA-DRB1 and-DQB1 alleles in Taiwanese Chinese are listed in Appendix 2. Table 5 shows the variable HLA-DQB1 alleles associated with the three most prevalently found alleles in HLA-DRB1 (DRB1*09:01, DRB1*12:02, and DRB1*15:01) in Taiwanese Chinese.

Human leukocyte antigen-A,-B, and-C haplotypes

Table 6 shows the 20 most commonly observed HLA-A,-B, and-C haplotypes in Taiwanese Chinese as predicted by the EM algorithm. Appendix 2 presents the list of HLA-A,-B, and-C haplotypes as predicted by the EM algorithm in this study.

Human leukocyte antigen-A,-B,-C, and-DRB1 haplotypes

The 20 most commonly recognized HLA-A,-B,-C, and-DRB1 haplotypes in Taiwanese Chinese as predicted by the EM algorithm are shown in Table 7. Appendix 2 shows the list of HLA-A,-B,-C, and-DRB1 haplotypes in this study as predicted by the EM algorithm.

Human leukocyte antigen-A,-B,-C,-DRB1, and-DQB1 haplotypes

In Table 8, the 20 most frequently observed HLA-A,-B,-C, -DRB1, and-DQB1 haplotypes in Taiwanese Chinese as predicted by the EM algorithm are presented. Appendix 2 shows the list of HLA-A,-B,-C,-DRB1, and-DQB1 haplotypes in this study as predicted by the EM algorithm.

Discussion

HLA molecules have been known as transplant antigens and have a strong relevance in tissue transplantation. The high-resolution SBT method can provide HLA allelic typing. High-resolution typing is advantageous for compatibility measurement between a potential stem cell donor and corresponding recipient.

In this study, a similar panel number of Taiwanese Chinese participants were tested for the HLA-A,-B, and-DRB1 alleles, and we found more individual HLA-B alleles than alleles in the HLA-A and-DRB1 loci. This is in agreement with studies in Chinese in the US donor registry, in a Chinese Han, and in a Taiwanese Chinese study reported by Gragert *et al*. [13], Li *et al*. [14], and Yang *et al*. [15], respectively. As a matter of fact, the same result was also observed in the 21 ethnic groups investigated in the US

Table 7: The 20 most commonly recognized human leukocyte antigen-A,-B,-C, and-DRB1 haplotypes in Taiwanese Chinese

A-B-C-DRB1 haplotype	Frequency	A-B-C-DRB1 haplotype	Frequency
33:03-58:01-03:02-03:01	0.04273	11:01-40:01-07:02-09:01	0.00957
02:07-46:01-01:02-09:01	0.04244	02:01-40:01-15:02-11:01	0.00916
11:01-15:02-08:01-12:02	0.02049	11:01-13:01-03:04-16:02	0.00913
30:01-13:02-06:02-07:01	0.02042	11:01-40:01-07:02-04:05	0.00861
33:03-58:01-03:02-13:02	0.01597	11:01-15:02-08:01-15:01	0.00796
11:01-13:01-03:04-15:01	0.01155	02:07-46:01-01:02-14:54	0.00681
02:03-38:02-07:02-16:02	0.01138	11:02-27:04-12:02-12:02	0.00662
11:01-40:01-07:02-08:03	0.01098	11:01-40:01-07:02-11:01	0.00657
11:01-46:01-01:02-09:01	0.01046	24:02-46:01-01:02-09:01	0.00613
02:07-46:01-01:02-08:03	0.00970	11:01-15:01-04:01-04:06	0.00610

Table 8: The 20 most frequently observed human leukocyte antigen-A,-B,-C,-DRB1, and-DQB1 haplotypes in Taiwanese Chinese

A-B-C-DRB1-DQB1 haplotype	Frequency	A-B-C-DRB1-DQB1 haplotype	Frequency
02:07-46:01-01:02-09:01-03:03	0.04192	11:01-40:01-07:02-09:01-03:03	0.01002
33:03-58:01-03:02-03:01-02:01	0.03985	11:01-46:01-01:02-09:01-03:03	0.00937
30:01-13:02-06:02-07:01-02:02	0.02467	11:01-13:01-03:04-16:02-05:02	0.00902
11:01-15:02-08:01-12:02-03:01	0.02259	02:07-46:01-01:02-14:54-05:02	0.00891
33:03-58:01-03:02-13:02-06:09	0.01744	11:01-15:02-08:01-15:01-06:01	0.00773
11:01-40:01-07:02-08:03-06:01	0.01318	11:01-40:01-07:02-04:05-04:01	0.00772
02:03-38:02-07:02-16:02-05:02	0.01139	11:01-51:01-14:02-09:01-03:03	0.00679
11:01-13:01-03:04-15:01-06:01	0.01135	02:01-40:01-07:02-09:01-03:03	0.00645
02:07-46:01-01:02-08:03-06:01	0.01116	24:02-46:01-01:02-09:01-03:03	0.00636
02:01-40:01-15:02-11:01-03:01	0.01003	11:01-13:01-03:04-12:02-03:01	0.00633

donor registry [13]. It is noteworthy that although 30 times more donors were tested in the current study than in our previous study, we found the 10–15 most prevalently observed alleles in each locus coincided with those in our previous study [15]. However, twice as many distinctive alleles were detected in each locus in this study than in the previous study [15]. Most significantly, as a result of employing the SBT typing method and the high number of donors tested, several novel alleles were discovered. These novel alleles include 23 HLA-A, 28 HLA-B, 6 HLA-C, and 13 HLA-DRB1 alleles [Table 9]. We suspect that these novel alleles are low-frequency alleles and are most likely restricted to those of Taiwanese Chinese ethnicity.

The five most commonly observed HLA-A alleles (A*11:01, A*24:02, A*02:07, A*02:01, and A*33:03),-B alleles (B*40:01, B*46:01, B*58:01, B*13:01, and B*15:02),-C alleles (C*07:02, C*01:02, C*03:04, C*08:01, and C*03:02) were identical in this population study and the US-Chinese population study [13]. However, only the three most prevalent alleles in the HLA-DRB1 locus (DRB1*09:01, DRB1*12:02, and DRB1*15:01) were identical between the two populations. The fourth and fifth most common alleles for the Taiwanese Chinese population were DRB1*08:03 and DRB1*11:01 and in the US-Chinese population were DRB1*03:01 and DRB1*08:03. These observations suggest that the population composition of HLA in Taiwanese Chinese and the US Chinese is similar but not identical.

From the point of view of LD, the diversity of alleles and allele associations was very obvious with the use of the molecular DNA typing method and the large number of donors tested in this study. Tables 3-5 show the genetic polymorphic features of association between HLA-A and-B, HLA-B and-C, and HLA-DRB1 and-DQB1 alleles. However, the 10–15 most commonly observed haplotypes of HLA-A-B-C, HLA-A-B-C-DRB1, and HLA-A-B-C-DRB1-DQB1 were similar in this study and the previous study [15]. This result indicates that investigating a panel with as many as 400 random donors is probably sufficient to determine the 10 to15 most common HLA haplotypes in Taiwanese.

We found that the eight most common HLA-A/B/C/DRB1/DQB1 haplotypes were identical in the US-Chinese [13] and the Taiwanese Chinese population reported here, but the 6th and the 10th most common in the US-Chinese population ranked 12th and 16th in the Taiwanese Chinese population. This finding reiterates the close similarity in HLA composition of the US and Taiwanese Chinese populations, but they are not absolutely identical. It also indicates that the US may have a broader mix of people from various regions of China than Taiwan.

In recent years, the concept of generating induced pluripotent stem cells (iPSC) using human somatic cells to possibly produce personalized pluripotent stem cell lines from individual patients sparked the hope of producing iPSC cell lines for transplantation therapies [16]. This approach could address rejection-associated issues concerning HLA matching of individual patients. However, the time and cost required for the generation of clinical-grade iPSC cell lines and the differentiated cell types for transplantation could limit its general application in clinical practice. A possibility to circumvent this hurdle is banking an inventory of iPSC cell lines with known HLAs, as was first proposed for the UK population [17]. However, this approach is hindered by HLA diversity since a large number of iPSC cell lines would be needed for the best HLA compatibility for recipients. An alternative strategy to decrease the necessity for a large number of cell lines is to establish a cell line bank with HLA homozygous cells [18]. With the large number of randomized donor cells studied at the high-resolution level in this study, we estimated the frequencies of homozygous HLA haplotypes in the Taiwanese Chinese population [Table 2]. We hope our data on HLA homozygosity in Taiwanese Chinese here may be useful as a reference for determining the number of iPSC cell lines needed to establish an HLA homozygous haplotype iPSC cell bank for the Taiwanese Chinese population for transplantation purposes.

Table 9: The 23 human leukocyte antigen-A, 28 human leukocyte antigen-B, 6 human leukocyte antigen-C, and 13 human leukocyte antigen-DRB1 novel alleles detected in Taiwanese Chinese in this study

HLA-A	HLA-B	HLA-C	HLA-DRB1
02:01:64	07:249	01:02:34	03:77
02:06:21	13:02:13	04:212	04:05:14
02:319	13:63N	07:341:02	04:05:15
02:466	15:01:37	07:375	04:178N
02:510	15:189	07:393N	04:207
02:541	15:327	07:486	08:71
02:570	27:86		09:01:08
02:575	35:307		10:04
02:586	39:77		12:01:06
02:610	40:01:44		14:84
02:614	40:137		15:02:11
11:01:69	40:158		15:116
11:119:02	40:159		16:16
11:165	40:221		
11:166	40:306		
11:167	40:326		
11:231	40:327		
11:235Q	46:01:20		
24:287	46:13:03		
24:333	46:60		
24:334	46:65		
24:334	51:112		
33:03:31	52:14		
	52:33		
	55:02:10		
	58:45:01		
	58:72N		
	58:77		

HLA: Human leukocyte antigen

Conclusion

We showed the increment number of HLA alleles at various loci and their variety and the number of allelic linkages between different loci. Further, we confirmed that the number of HLA haplotype combinations for a given population is directly proportional to the number of unrelated random donors tested and the level of HLA typing resolution achieved. In addition, novel HLA alleles revealed in a population study may inspire appreciation and trigger curiosity about the uniqueness and polymorphic nature of the HLA genetic system.

Declaration of patient consent

The authors certify that all patients provided appropriate patient consent forms. In the form, all patients gave consent for their images and other clinical information to be reported in the journal. All patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Gallo S, Woolfrey AE, Burroughs LM, Storer BE, Flowers ME, Hari P, et al. Marrow grafts from HLA-identical siblings for severe aplastic anemia: Does limiting the number of transplanted marrow cells reduce the risk of chronic GvHD? Bone Marrow Transplant 2016;51:1573-8.
2. Liu Z, Chen G, Kang X, Han M, Chen R, Chen C, et al. A multiplex allele-specific real-time polymerase chain reaction assay for HLA-B*13:01 genotyping in four Chinese populations. HLA 2016;88:164-71.
3. Erlich HA, Opelz G, Hansen J. HLA DNA typing and transplantation. Immunity 2001;14:347-56.
4. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The IPD and IMGT/HLA database: Allele variant databases. Nucleic Acids Res 2015;43:D423-31.
5. Kempenich JH, Setterholm M, Maiers M. Haplotype associations of 90 rare alleles from the National Marrow Donor Program. Tissue Antigens 2006;67:284-9.
6. Maiers M, Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population. Hum Immunol 2007;68:779-88.
7. Wen SH, Lai MJ, Yang KL. Human leukocyte antigen-A, -B, and -DRB1 haplotypes of cord blood units in the Tzu Chi Taiwan Cord Blood Bank. Hum Immunol 2008;69:430-6.
8. Vorechovsky I, Kralovicova J, Laycock MD, Webster AD, Marsh SG, Madrigal A, et al. Short tandem repeat (STR) haplotypes in HLA: An integrated 50-kb STR/linkage disequilibrium/gene map between the RING3 and HLA-B genes and identification of STR haplotype diversification in the class III region. Eur J Hum Genet 2001;9:590-8.
9. Pédron B, Yakouben K, Guérin V, Borsali E, Auvrignon A, Landman J, et al. HLA alleles and haplotypes in French North African immigrants. Hum Immunol 2006;67:540-50.
10. Chen MJ, Chu CC, Shyr MH, Lin CL, Lin PY, Yang KL. A novel HLA-B allele, B*5214, detected in a Taiwanese volunteer bone marrow donor using a sequence-based typing method. Int J Immunogenet 2010;37:39-41.
11. Chen MJ, Yang TC, Chu CC, Shyr MH, Lin CL, Lin PY, et al. Detection of a novel HLA-B27 allele, B*2740, in Taiwanese volunteer bone marrow donors by sequence-based typing: Curiosity rewarded. Int J Immunogenet 2009;36:207-11.
12. Excoffier L, Lischer HE. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 2010;10:564-7.
13. Gragert L, Madbouly A, Freeman J, Maiers M. Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing for the entire US donor registry. Hum Immunol 2013;74:1313-20.
14. Li XF, Zhang X, Chen Y, Zhang KL, Liu XJ, Li JP. An analysis of HLA-A, -B, and -DRB1 allele and haplotype frequencies of 21,918 residents living in Liaoning, China. PLoS One 2014;9:e93082.
15. Yang KL, Chen SP, Shyr MH, Lin PY. High-resolution human leukocyte antigen (HLA) haplotypes and linkage disequilibrium of HLA-B and -C and HLA-DRB1 and -DQB1 alleles in a Taiwanese population. Hum Immunol 2009;70:269-76.
16. de Rham C, Villard J. Potential and limitation of HLA-based banking of human pluripotent stem cells for cell therapy. J Immunol Res 2014;2014:518135.
17. Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell 2012;11:147-52.
18. Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and iPS cells. Nat Biotechnol 2008;26:739-40.