ESC HEART FAILURE
ESC Heart Failure 2019; **6**: 388–395
Published online 23 January 2019 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12406

ORIGINAL RESEARCH ARTICLE

Determination of HLA-A, -B, -C, -DRB1 and -DQB1 allele and haplotype frequencies in heart failure patients

Santiago Roura[1,2†], Francesc Rudilla[3,4†], Paloma Gastelurrutia[1,2], Emma Enrich[3,4], Eva Campos[3], Josep Lupón[2,5,6], Evelyn Santiago-Vacas[5], Sergi Querol[4,7]* and Antoni Bayés-Genís[1,2,5,6]*

[1]*ICREC Research Program, Germans Trias i Pujol Health Science Research Institute, Badalona, Spain;* [2]*CIBER Cardiovascular, Instituto de Salud Carlos III, Madrid, Spain;* [3]*Histocompatibility and Immunogenetics Laboratory, Blood and Tissue Bank, Barcelona, Spain;* [4]*Transfusional Medicine Group, Vall d'Hebron Research Institute, Autonomous University of Barcelona (UAB), Barcelona, Spain;* [5]*Cardiology Service, Germans Trias i Pujol University Hospital, Badalona, Spain;* [6]*Department of Medicine, UAB, Barcelona, Spain;* [7]*Cell Therapy Unit, Blood and Tissue Bank, Barcelona, Spain*

Abstract

Aims Cell therapy can be used to repair functionally impaired organs and tissues in humans. Although autologous cells have an immunological advantage, it is difficult to obtain high cell numbers for therapy. Well-characterized banks of cells with human leukocyte antigens (HLA) that are representative of a given population are thus needed. The present study investigates the HLA allele and haplotype frequencies in a cohort of heart failure (HF) patients.

Methods and results We carried out the HLA typing and the allele and haplotype frequency analysis in 247 ambulatory HF patients. We determined HLA class I (A, B, and C) and class II (DRB1 and DQB1) using next-generation sequencing technology. The allele frequencies were obtained using Python for Population Genomics (PyPop) software, and HLA haplotypes were estimated using HaploStats. A total of 30 HLA-A, 56 HLA-B, 23 HLA-C, 36 HLA-DRB1, and 15 HLA-DQB1 distinct alleles were identified within the studied cohort. The genotype frequencies of all five HLA loci were in Hardy–Weinberg equilibrium. We detected differences in HLA allele frequencies among patients when the etiological cause of HF was considered. There were a total of 494 five-loci haplotypes, five of which were present six or more times. Moreover, the most common estimated HLA haplotype was HLA-A*01:01, HLA-B*08:01, HLA-C*07:01, HLA-DRB1*03:01, and HLA-DQB1*02:01 (6.07% haplotype frequency per patient). Remarkably, the 11 most frequent haplotypes would cover 31.17% of the patients of the cohort in need of allogeneic cell therapy.

Conclusions Our findings could be useful for improving allogeneic cell administration outcomes without concomitant immunosuppression.

Keywords Allogeneic cell therapy; Allele frequency; Haplotype frequency; Heart failure

Received: 27 June 2018; Accepted: 12 December 2018
**Correspondence to: Antoni Bayés-Genís, MD, PhD, FESC, FHFA, Head, Heart Institute, Hospital Universitari Germans Trias i Pujol, and Department of Medicine, UAB, Carretera del Canyet s/n 08916 Badalona, Spain. Tel: +34 934978915; Fax: +34 934978939. Email: abayesgenis@gmail.com*
Sergio Querol, MD, PhD, Head of Cell Therapy Services, Catalan Blood and Tissue Bank (BST), Frederic Duran i Jordà Center, Barcelona, Spain. Tel: +34 935573505; Fax: +34 935573506. Email: squerol@bst.cat
†These authors contributed equally to the present work.

Introduction

Heart failure (HF) is recognized as the true epidemic of the 21st century, affecting 1–2% of the adult population in developed countries, with a prevalence of ≥10% among those aged 70 years and older.[1] Despite effective medication, left ventricular assist devices, and surgeries that have improved patient survival and comorbidities, replacement of the irreparably injured heart by a donor organ is the only option in some cases. However, this is often impaired by limited donors, organ rejection, and receptors that need chronic immunosuppression, which greatly reduces the patient's quality of life.[2] In this context, cell-based therapy constitutes a promising option to restore damaged heart tissue.[3,4] Autologous cell

transplantation is preferred for regenerative purposes because there is no risk of immune rejection. However, the use of autologous cells has some potential limitations. In general, high cell numbers are not immediately available from the same recipient. Moreover, cells isolated from elderly donors show decreased differentiation and regenerative capabilities, resulting in disappointing treatment outcomes. To overcome these limitations, therapeutic strategies are using allogeneic cells despite the need for human leukocyte antigen (HLA) matching between the donor and recipient.

Histocompatibility is mainly determined by the major histocompatibility complex genes (termed HLA in humans), the most polymorphic genes in the human genome, which are located on a 3.6 Mb region on chromosome 6p21. The HLA genes have important roles in immune system regulation.[5–7] Mainly, the HLA molecules present antigens to T lymphocytes that direct the immune response and prevent autoreactivity. It is important to note that the immune response against HLA antigens is a major obstacle in organ and haematopoietic stem cell transplantation. Currently, HLA typing is used to search for an adult HLA match or cord blood donors to prevent graft-versus-host disease and graft rejection during transplantation.[8–12] Thus, to increase the success of the functional engraftment of a variety of cell suspensions or cell-embedded implants, there is a need for ready-to-use banks of HLA homozygous stem cells covering the largest number of HLA combinations.

In this field of study, next-generation sequencing (NGS) approaches are based on amplification of the selected regions by PCR followed by massively parallel sequencing of the amplicons. In recent years, different HLA typing strategies by NGS have been developed that provide high throughput sequencing, unambiguous high-resolution results, and reduced costs per sample.[13–16]

Thus, the aim of this study was to perform a high-resolution exploratory HLA analysis in a cohort of patients with HF to determine the HLA-A, -B, -C, -DRB1, and -DQB1 allele and haplotype frequencies by NGS and develop conveniently designed donor panels. These data should maximize the match probabilities for this patient population and improve potential allogeneic cell therapy outcomes.

Table 1 Baseline demographic, clinical, and biochemical data of the study participants

	n = 247
Age (years)	68.7 ± 12.2
Male sex	209 (84.6%)
Aetiology	
Ischaemic heart disease	152 (61.5%)
Dilated CM	42 (17%)
Hypertensive CM	9 (3.6%)
Alcoholic CM	12 (4.9%)
Drug-induced CM	4 (1.6%)
Valvular disease	13 (5.3)
Hypertrophic CM	5 (2.0%)
Other	10 (4%)
HF duration (months)	60 (14–119)
LVEF	29.5% ± 6.5
NYHA functional class	
I	8 (3.2%)
II	156 (63.2%)
III	81 (32.8%)
IV	2 (0.8%)
Co-morbidities	
Hypertension	182 (73.7%)
Diabetes mellitus	112 (45.3%)
COPD	71 (28.7%)
Renal failure[a]	146 (59.1%)
Anaemia[b]	116 (47%)
Atrial fibrillation/flutter	100 (40.5%)
Biochemical	
Na	139 ± 3.9
Haemoglobin	12.9 ± 1.6
eGFR	55.5 ± 25.2
NTproBNP	1673 (702–4115)
Treatments	
ACEI/ARB	207 (83.8%)
Beta-blockers	234 (94.7%)
MRA	171 (69.2%)
Loop diuretics	214 (86.6%)
Digoxin	70 (28.3%)
Ivabradine	38 (15.4%)
Statins	199 (80.6%)
ICD	83 (33.6%)
CRT	51 (20.6%)

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CM, cardiomyopathy; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter device; LVEF, left ventricular ejection fraction; MRA: mineral corticoid receptor antagonist; NYHA, New York Heart Association.
Data expressed as mean ± standard deviation, median (25th–75th percentiles), or absolute number (percentage).
[a]eGFR (CKD-EPI) <60 mL/min/1.73 m^2.
[b]Hb < 12 g/dL in women and <13 g/dL in men.

Methods

Study population

Our study cohort included 247 ambulatory patients who attended a multidisciplinary HF unit (*Table 1*). The referral inclusion criteria are described elsewhere.[17,18] All patients attended follow-up visits at regular predefined intervals and additional visits when required in cases of decompensation. Each subject provided their written informed consent prior to participation. The study protocol was approved by the Clinical Research Ethics Committee of our institution (reference number PI-17-044) and was designed in accordance with the principles outlined in the 2013 revision of the Declaration of Helsinki of 1975.[19] Patients provide specific ancestry data.

Blood extraction and processing

Blood samples (~3 mL) were collected into EDTA tubes via standard forearm venipuncture performed between 9:00 a.m.

and 11:00 a.m. and were processed within 4 h after collection. Samples and data from patients included in this study were processed and collected by the IGTP-HUGTP Biobank from the Spanish National Biobanks Network of Instituto de Salud Carlos III (PT13/0010/0009) and Tumour Bank Network of Catalonia. All laboratory measurements were performed by staff blinded to the patients' clinical characteristics. DNA was extracted from peripheral blood using the QIAsymphony DNA purification system (Qiagen, Toronto, Canada) according to the manufacturer's protocol.

Table 2 Class I allele frequencies in heart failure patients

HLA-A	*N*	AF	HLA-B	*N*	AF	HLA-C	*N*	AF
*A*02:01*	119	24.09	*B*44:03*	46	9.31	*C*07:01*	66	13.36
*A*01:01*	66	13.36	*B*18:01*	37	7.49	*C*04:01*	64	12.96
*A*24:02*	50	10.12	*B*44:02*	36	7.29	*C*05:01*	58	11.74
*A*03:01*	46	9.31	*B*08:01*	31	6.28	*C*06:02*	47	9.51
*A*29:02*	35	7.09	*B*51:01*	31	6.28	*C*16:01*	38	7.69
*A*11:01*	31	6.28	*B*07:02*	27	5.47	*C*07:02*	33	6.68
*A*26:01*	19	3.85	*B*14:02*	21	4.25	*C*12:03*	32	6.48
*A*30:02*	15	3.04	*B*38:01*	18	3.64	*C*08:02*	24	4.86
*A*32:01*	14	2.83	*B*35:01*	18	3.64	*C*01:02*	22	4.45
*A*31:01*	12	2.43	*B*50:01*	17	3.44	*C*02:02*	21	4.25
*A*23:01*	10	2.02	*B*15:01*	16	3.24	*C*03:03*	17	3.44
*A*68:01*	9	1.82	*B*57:01*	14	2.83	*C*15:02*	14	2.83
*A*33:01*	8	1.62	*B*49:01*	14	2.83	*C*03:04*	14	2.83
*A*68:02*	8	1.62	*B*45:01*	13	2.63	*C*12:02*	9	1.82
*A*25:01*	8	1.62	*B*27:05*	12	2.43	*C*14:02*	7	1.42
*A*30:01*	8	1.62	*B*40:01*	11	2.23	*C*17:01*	7	1.42
*A*02:05*	7	1.42	*B*52:01*	10	2.02	*C*15:05*	6	1.21
*A*33:03*	5	1.01	*B*35:03*	10	2.02	*C*03:02*	3	0.61
*A*66:01*	5	1.01	*B*53:01*	9	1.82	*C*05:09*	3	0.61
*A*03:02*	4	0.81	*B*40:02*	7	1.42	*C*02:10*	3	0.61
*A*02:02*	3	0.61	*B*37:01*	7	1.42	*C*07:04*	3	0.61
*A*69:01*	3	0.61	*B*39:01*	7	1.42	*C*16:02*	2	0.40
*A*24:03*	2	0.40	*B*07:05*	6	1.21	*C*15:06*	1	0.20
*A*74:03*	1	0.20	*B*35:02*	6	1.21			
*A*02:03*	1	0.20	*B*58:01*	6	1.21			
*A*26:08*	1	0.20	*B*13:02*	6	1.21			
*A*30:04*	1	0.20	*B*56:01*	5	1.01			
*A*01:02*	1	0.20	*B*41:01*	4	0.81			
*A*29:01*	1	0.20	*B*35:08*	4	0.81			
*A*24:10*	1	0.20	*B*39:06*	4	0.81			
			*B*41:02*	4	0.81			
			*B*15:03*	3	0.61			
			*B*15:17*	3	0.61			
			*B*14:01*	3	0.61			
			*B*55:01*	3	0.61			
			*B*44:05*	3	0.61			
			*B*40:06*	2	0.40			
			*B*50:02*	2	0.40			
	494	100.00					494	100.00
			*B*51:08*	1	0.20			
			*B*39:24*	1	0.20			
			*B*15:09*	1	0.20			
			*B*27:03*	1	0.20			
			*B*18:03*	1	0.20			
			*B*57:03*	1	0.20			
			*B*51:02*	1	0.20			
			*B*78:01*	1	0.20			
			*B*35:30*	1	0.20			
			*B*47:01*	1	0.20			
			*B*27:12*	1	0.20			
			*B*15:24*	1	0.20			
			*B*27:02*	1	0.20			
			*B*44:130*	1	0.20			
			*B*15:16*	1	0.20			
			*B*15:18*	1	0.20			
			*B*46:01*	1	0.20			
			*B*39:02*	1	0.20			
				494	100.00			

AF, allelic frequency; *N*, number of observed alleles.

Human leukocyte antigen typing

HLA-A, -B, -C, -DRB1, and -DQB1 were analysed by NGS using the Illumina MiSeq instrument. Focusing on key exons, the analysis characterized HLA exons 2 to 4 for class I alleles and exons 2 and 3 for class II alleles using the NGSengine software (version 2.8.0, GenDX, Netherlands) and the November 2017 IPD-IMGT/HLA database 3.30.0, as the allele reference library. The HLA allele frequencies were estimated using PyPop software (version http://www.pypop.org).

Human leukocyte antigen haplotype determination

We determined the HLA haplotype by complete typing of HLA-A, -B, -C, -DRB1, and -DQB1. The haplotypes were not determined by segregation because no parents' samples were available. The HLA haplotype estimation was performed using HaploStats (http://www.haplostats.org) software based on haplotype frequencies. The haplotypes were counted using Microsoft Excel. The exact test for deviation from Hardy–Weinberg Equilibrium was evaluated by PyPop software, which uses an algorithm to estimate the *P*-value. Also, PyPop analyses were performed to estimate the haplotype, linkage disequilibrium (D), and relative linkage disequilibrium (D').

Results

Table 1 shows the clinical characteristics of the studied population. In general, patients were middle-aged and predominantly male, frequently had an ischaemic aetiology, were in NYHA functional class II or III, and were treated following contemporary guidelines. Within the cohort of HF patients, we measured the HLA-A, -B, -C, -DRB1, and -DQB1 allele and haplotype frequencies using whole genomic DNA extracted from peripheral blood samples and high-resolution NGS technology. While HLA class I alleles were defined based on the sequences of exons 2, 3, and 4, HLA class II alleles were designated based on the sequences of exons 2 and 3. Once HLA typing was performed, we noticed that all patients were heterozygous at the five HLA loci. Furthermore, the genotype frequencies of all five HLA loci were in Hardy–Weinberg equilibrium proportions.

In this patient cohort, the number of distinct HLA alleles were 30 for HLA-A, 56 for HLA-B, 23 for HLA-C, 36 for HLA-DRB1, and 15 for HLA-DQB1. Three HLA-A alleles, A*02:01 (24.09%), A*01:01 (13.36%), and A*24:02 (10.12%), were the most frequent HLA-A alleles. Eleven of the HLA-A alleles identified (36.66%) appeared less than 1%. With regard to HLA-B, B*44:03 (9.31%), B*18:01 (7.49%), B*44:02 (7.29%), B*08:01 (6.28%), B*51:01 (6.28%), and B*07:02 (5.47%) exhibited the highest frequencies. Twenty-nine of the HLA-B alleles (32%) appeared less than 1%. As for HLA-C, three alleles, C*07:01 (13.36%), C*04:01 (12.96%), and C*05:01 (11.74%), exhibited the most frequencies. Almost one-quarter of the HLA-C alleles appeared with a frequency less than 1% (*Table 2*).

The most likely HLA-DRB1 alleles were DRB1*07:01 (14.78%) and DRB1*03:01 (13.16%). Eleven HLA-DRB1 alleles (22.22%) were observed less than 1%. The most frequent alleles were DQB1*03:01 (15.38%), DQB1*05:01 (14.57%), DQB1*02:01 (13.36%), and DQB1*02:02 (13.36%). All HLA-DQB1 alleles appeared six or more times (*Table 3*).

Importantly, the most frequent HLA alleles in this HF population are in line with the most frequent HLA alleles presented in our reference population. In particular, we used as reference database all NGS-typed samples collected in the bank of samples of Blood and Tissue Bank (Barcelona, Spain). Because Blood and Tissue Bank implemented NGS for typing their samples in 2015, they have assessed more than 15 000 samples from our area (data not shown). Also,

Table 3 Class II allele frequencies in heart failure patients

HLA-DRB1	*N*	AF	HLA-DQB1	*N*	AF
*DRB1*07:01*	73	14.78	*DQB1*03:01*	76	15.38
*DRB1*03:01*	65	13.16	*DQB1*05:01*	72	14.57
*DRB1*15:01*	40	8.10	*DQB1*02:01*	66	13.36
*DRB1*13:01*	36	7.29	*DQB1*02:02*	66	13.36
*DRB1*01:01*	35	7.09	*DQB1*03:02*	48	9.72
*DRB1*11:01*	27	5.47	*DQB1*06:02*	38	7.69
*DRB1*11:04*	23	4.66	*DQB1*06:03*	36	7.29
*DRB1*01:02*	21	4.25	*DQB1*03:03*	22	4.45
*DRB1*04:04*	17	3.44	*DQB1*04:02*	15	3.04
*DRB1*13:02*	17	3.44	*DQB1*05:03*	13	2.63
*DRB1*04:05*	13	2.63	*DQB1*05:02*	12	2.43
*DRB1*09:01*	11	2.23	*DQB1*06:04*	9	1.82
*DRB1*11:02*	10	2.02	*DQB1*06:09*	8	1.62
*DRB1*04:02*	10	2.02	*DQB1*06:01*	7	1.42
*DRB1*16:01*	10	2.02	*DQB1*03:19*	6	1.21
*DRB1*08:01*	10	2.02			
*DRB1*04:03*	9	1.82			
*DRB1*10:01*	9	1.82			
*DRB1*15:02*	8	1.62			
*DRB1*14:54*	7	1.42			
*DRB1*04:01*	6	1.21			
*DRB1*13:03*	6	1.21			
*DRB1*04:07*	5	1.01			
*DRB1*01:03*	5	1.01			
*DRB1*12:01*	5	1.01			
*DRB1*14:04*	4	0.81			
*DRB1*04:06*	2	0.40			
*DRB1*13:05*	2	0.40			
*DRB1*16:02*	1	0.20			
*DRB1*15:03*	1	0.20			
*DRB1*04:11*	1	0.20			
*DRB1*14:05*	1	0.20			
*DRB1*13:11*	1	0.20			
*DRB1*08:04*	1	0.20			
*DRB1*04:37*	1	0.20			
*DRB1*12:02*	1	0.20			
	494	100.00		494	100.00

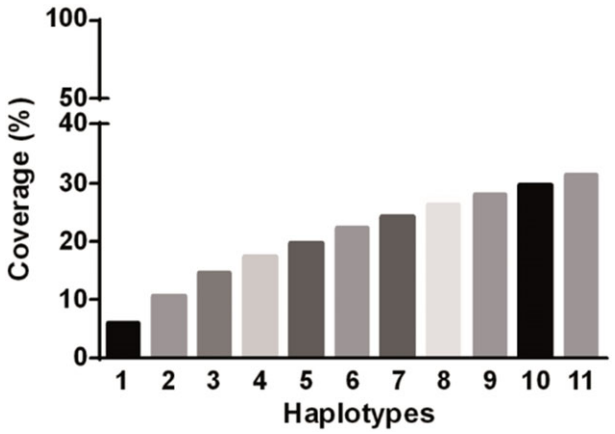
AF, allele frequency; *N*, number of observed alleles.

Table 4 The most frequent haplotypes in heart failure patients

ID	Haplotypes	*N*	HF	PHF
1	*A*01:01, B*08:01, C*07:01, DRB1*03:01, DQB1*02:01*	15	3.04	6.07
2	*A*29:02, B*44:03, C*16:01, DRB1*07:01, DQB1*02:02*	11	2.23	4.45
3	*A*30:02, B*18:01, C*05:01, DRB1*03:01, DQB1*02:01*	10	2.02	4.05
4	*A*23:01, B*44:03, C*04:01, DRB1*07:01, DQB1*02:02*	7	1.42	2.83
5	*A*02:01, B*08:01, C*07:01, DRB1*03:01, DQB1*02:01*	6	1.21	2.43
6	*A*33:01, B*14:02, C*08:02, DRB1*01:02, DQB1*05:01*	6	1.21	2.43
7	*A*02:01, B*18:01, C*05:01, DRB1*03:01, DQB1*02:01*	5	1.01	2.02
8	*A*02:01, B*44:02, C*05:01, DRB1*01:01, DQB1*05:01*	5	1.01	2.02
9	*A*02:01, B*07:02, C*07:02, DRB1*15:01, DQB1*06:02*	4	0.81	1.62
10	*A*24:02, B*45:01, C*16:01, DRB1*10:01, DQB1*05:01*	4	0.81	1.62
11	*A*02:01, B*44:02, C*05:01, DRB1*11:01, DQB1*03:01*	4	0.81	1.62
				31.17

HF, haplotype frequency; ID, identification; *N*, number of observed haplotypes; PHF, population haplotype frequency.

Figure 1 The 11 most frequent haplotypes are present in the 31.17% of the patients of our cohort. Each column represents the accumulated percentage of individuals that have each haplotype (referenced in *Table 4* as ID) and its previous ones.



we used the Allele Frequency Net Database as a reference (http://allelefrequencies.net).

We likewise studied the HLA allele, taking into account the two main etiological causes of HF in our cohort of patients. Of note, the cohorts of patients with dilated cardiomyopathy (DCM) and ischaemic HF (IHF) showed some differences on HLA allele frequencies. Regarding DCM patients, HLA-A*03:01 (5.95%), HLA-B*08:01 (1.19%), and HLA-DRB1*03:01 (8.33%) were less represented as compared with the IHF group and reference database. On the contrary, HLA-C*04:01 (20.24%), HLA-DRB1*04:03 (3.57%), HLA-DRB1*04:04 (4.76%), and HLA-DRB1*04:05 (3.57%) were more common on patients with DCM. On the other hand, the IHF patients had over represented HLA-DQB1*05:01 (16.45%) and HLA-C*05:01 (12.50%). HLA-DQB1*03:01 (12.50%) was also less expressed in this cohort of patients.

When ethnicity of our cohort of HF patients was considered, we identified 383 unique haplotypes from the 494 five-loci haplotypes analysed, five of which were present six or more times (*Table S1*). The most common five-loci haplotype detected was A*01:01-B*08:01-C*07:01-DRB1*03:01-DQB1*02:01 (3.04%), which had a haplotype frequency of 6.07% per patient (*Table 4*). Remarkably, 31.17% of patients would be covered by the 11 most frequent HLA haplotypes (*Figure 1*).

Lastly, we focused on the two main etiological causes (DCM and IHF) (*Tables S2–S7*). In this context, we found that the frequent haplotype A*29:02-B*44:03-C*16:01-DRB1*07:01-DQB1*02:02 was not present in any of the DCM patients. Moreover, the haplotype A*01:01-B*08:01-C*07:01-DQB1*02:01-DRB1*03:01 (1.19%) was found only once.

Discussion

The increased incidence and prevalence of congestive HF have led to the need for novel treatment strategies, including cell-based therapies. Translating cell administration into the clinic requires the ability to deliver a safe and efficacious product that is ready-to-use at the optimal dosage. Allogeneic cell therapy is immediately available and provides a high number of cells[20]; however, it is clearly a disruptive concept in biology. The standard immunologic dogma holds that any foreign tissue will elicit an immune reaction.[20] This is clearly apparent in whole organ, tissue, or cell transplants in which aggressive immunosuppression is necessary to protect allografts from rejection.[21] Thus, the analysis of histocompatibility is most relevant in this field, where differences between the donor's HLA alleles and the recipient's trigger the immune system to reject the transplant. The World Marrow Donor Association guidelines for establishing the extent and quality of histocompatibility testing for unrelated donor registries, umbilical cord blood banks, and transplant centres involved an international exchange of cells for allogeneic therapy.[22]

As the field of cell-based therapy evolves, it has become evident that various cell types – mesenchymal stem cells (MSCs) being the prototype – have sufficient ability to evade and/or suppress the immune system to the extent that they may be used as allografts without requiring concomitant immunosuppression.[23,24] Moreover, increasing evidence has suggested that the effect of infused MSCs is not direct, but rather due to paracrine signalling. Paracrine signalling is consistent with findings in which a low number of retained or seeded cells could promote restorative effects, such as forming vessels to protect resident cardiomyocytes from apoptosis, and mobilize host stem or progenitor cells to potentiate both vascularization and cardiomyogenesis.[25,26] Thus, alternative cell sources are being examined, including pluripotent stem cells (iPS). The iPS cells are in the preclinical stage, can be generated after inducing the expression of transcription factors associated with pluripotency, and exhibit unlimited self-renewal and differentiation to many cell lineage types.[27,28] Clinically, the pluripotency state of iPS allows for a wide range of disease treatments, and their predifferentiation *ex vivo* may guarantee their safeness.[29–31] Nevertheless, their high proliferation rate increases the risks associated with products containing iPS (e.g. risk of tumour formation).[32] Indeed, in contrast to MSCs, derivatives from iPS can currently only be used for autologous cell administration. Thus, it is necessary to generate iPS banks for HLA-matched allogeneic cell therapy based on known donor and recipient HLA types. Moreover, the major purposes of developing these iPS banks are to ensure cost-effectiveness, solve the issue of high time consumption in processing autologous iPS or derivatives, and guarantee their utility for acute patients.[33]

The presented results showed that the most common five-loci haplotype detected was A*01:01-B*08:01-C*07:01-DRB1*03:01-DQB1*02:01 (3.04%), which had a haplotype frequency of 6.07% per patient in our cohort. This HLA-estimated haplotype within our cohort of patients is common and conserved in North European Caucasians.[34–36] In particular, when we consider the Allele Frequency Net Database, the haplotype frequency of A*01:01-B*08:01-C*07:01-DQB1*02:01-DRB1*03:01 in North European Caucasians is over 3%. Remarkably, 31.17% of patients in our cohort would be covered by the 11 most frequent haplotypes. This finding from a real setting is in agreement with that previously reported by Gourraud *et al*.[37] These authors developed a mathematical model and calculated that, in order to obtain iPS for the 20 most frequent HLA haplotypes, 26 000 European-American donors would need to be analysed and 50% would be compatible. This confirms that relatively few, but very well selected, donors would give rise to iPS lines with a very important clinical utility. To carry out the screening and identify the largest number of possible donors, it would be necessary to collaborate with multiple centres worldwide.[38] This study searched among potential bone marrow donors and samples preserved in umbilical cord banks, because both are already HLA typed. The findings from this study will be part of a subsequent study of samples stored in the Catalan Blood and Tissue Bank (BST). The search and selection of HLA homozygous cord units for the 11 most frequent haplotypes could extend future administrations of therapeutic cells.

Remarkably, despite further studies using a large cohort of HF patients are warranted to potentially assess statistical significances, our findings suggest a protective role for some haplotypes such as HLA-B*08:01 and HLA-DQB1*03:01, which are underrepresented in DCM and IHF patients, respectively. In contrast, the haplotypes HLA-DRB1*04:03, HLA-DRB1*04:04, and HLA-DRB1*04:05 could be associated with DCM because their frequencies are higher compared with IHF patients and control subjects (reference database).

In sum, the present study revealed, for the first time, the most frequent HLA allele combinations within a cohort of ambulatory HF patients. Autologous administration of MSC and iPS is still preferred for regenerative purposes because the rejection risks are avoided. However, our findings suggest that safe iPS-based products, e.g. predifferentiated cardiomyocytes or endothelial cells, can be useful for treating these patients in future clinical trials if the recipient and donor are HLA matched. Indeed, an iPS bank of 11 cell lines will cover almost one-third of this patient population.

Acknowledgements

We specially thank the nurses, Beatriz González, Margarita Rodríguez, Carmen Rivas, Violeta Díaz, Núria Benito, Albas Ros, Jessica Ruiz, and Jenifer García, for data collection and their invaluable work at the HF unit.

Conflict of interest

None declared.

Funding

This study was supported by Grants from the Ministerio de Educación y Ciencia (SAF2014-59892), Fundació La MARATÓ de TV3 (201502, 201516), CIBER Cardiovascular (CB16/11/00403), Generalitat de Catalunya 2017SGR 00483, CERCA Programme and PERIS Programme, Departament de Salut SLT002/16/00234, SLT002_16_00209, and AdvanceCat with the support of ACCIÓ (Catalonia Trade & Investment; Generalitat de Catalunya) under the Catalonian European Regional Development Fund operational programme, 2014–2020.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Haplotype frequencies in heart failure patients.
Table S2. Class I allele frequencies in dilated cardiomyopathy patients.
Table S3. Class II allele frequencies in dilated cardiomyopathy patients.
Table S4. Class I allele frequencies in ischemic heart failure patients.
Table S5. Class II allele frequencies in ischemic heart failure patients.
Table S6. Haplotype frequencies in dilated cardiomyopathy patients.
Table S7. Haplotype frequencies in ischemic heart failure patients.

References

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. Authors/Task Force Members; Document Reviewers. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016; **18**: 891–975.
2. Söderlund C, Rådegran G. Immunosupressive therapies after heart transplantation – the balance between under- and over-immunosuppression. *Transplant Rev (Orlando)* 2015; **29**: 181–189.
3. Terzic A, Behfar A. Stem cell therapy for heart failure: ensuring regenerative proficiency. *Trends Cardiovasc Med* 2016; **26**: 395–404.
4. Bayés-Genís A, Gálvez-Montón C, Roura S. Cardiac tissue engineering: lost in translation or ready for translation? *J Am Coll Cardiol* 2016; **68**: 724–726.
5. Klein J, Sato A. The HLA system. First of two parts. *N Engl J Med* 2000; **343**: 702–709.
6. Klein J, Sato A. The HLA system. Second of two parts. *N Engl J Med* 2000; **343**: 782–786.
7. McDevitt HO. Discovering the role of the major histocompatibility complex in the immune response. *Annu Rev Immunol* 2000; **18**: 1–17.
8. Afzali B, Lechler RI, Hernandez-Fuentes MP. Allorecognition and the alloresponse: clinical implications. *Tissue Antigens* 2007; **69**: 545–556.
9. Opelz G, Döhler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. *Transplantation* 2007; **84**: 137–143.
10. Petersdorf EW. Optimal HLA matching in hematopoietic cell transplantation. *Curr Opin Immunol* 2008; **20**: 588–593.
11. Spellman SR, Eapen M, Logan BR, Mueller C, Rubinstein P, Setterholm MI, Woolfrey AE, Horowitz MM, Confer DL, Hurley CK. A perspective on the selection of unrelated donors and cord blood units for transplantation. *Blood* 2012; **120**: 259–265.
12. Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. *Transplantation* 2012; **93**: 751–756.
13. Hosomichi K, Shiina T, Tajima A, Inoue I. The impact of next-generation sequencing technologies on HLA research. *J Hum Genet* 2015; **60**: 665–673.
14. Erlich HA. HLA typing using next generation sequencing: an overview. *Hum Immunol* 2015; **76**: 887–890.
15. Duke JL, Lind C, Mackiewicz K, Ferriola D, Papazoglou A, Gasiewski A, Heron S, Huynh A, McLaughlin L, Rogers M, Slavich L, Walker R, Monos DS. Determining performance characteristics of an NGS-based HLA typing method for clinical applications. *HLA* 2016; **87**: 141–152.
16. Carapito R, Radosavljevic M, Bahram S. Next-generation sequencing of the HLA locus: methods and impacts on HLA typing, population genetics and disease association studies. *Hum Immunol* 2016; **77**: 1016–1023.
17. Zamora E, Lupón J, Vila J, Urrutia A, de Antonio M, Sanz H, Grau M, Ara J, Bayés-Genís A. Estimated glomerular filtration rate and prognosis in heart failure: value of the Modification of Diet in Renal Disease Study-4, chronic kidney disease epidemiology collaboration, and Cockroft-Gault formulas. *J Am Coll Cardiol* 2012; **59**: 1709–1715.
18. Gastelurrutia P, Lupón J, de Antonio M, Urrutia A, Díez C, Coll R, Altimir S, Bayes-Genis A. Statins in heart failure: the paradox between large randomized clinical trials and real life. *Mayo Clin Proc* 2012; **87**: 555–560.
19. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013; **310**: 2191–2194.
20. Karantalis V, Schulman IH, Balkan W, Hare JM. Allogeneic cell therapy: a new paradigm in therapeutics. *Circ Res* 2015; **116**: 12–15.
21. Chinen J, Buckley RH. Transplantation immunology: solid organ and bone marrow. *J Allergy Clin Immunol* 2010; **125**: S324–S333.
22. Bochtler W, Maiers M, Oudshoorn M, Marsh SG, Raffoux C, Mueller C, Hurley CK. World Marrow Donor Association guidelines for use of HLA nomenclature and its validation in the data exchange among hematopoietic stem cell donor registries and cord blood banks. *Bone Marrow Transplant* 2007; **39**: 737–741.
23. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol* 2014; **32**: 252–260.
24. van den Akker F, de Jager SC, Sluijter JP. Mesenchymal stem cell therapy for cardiac inflammation: immunomodulatory properties and the influence of toll-like receptors. *Mediators Inflamm* 2013; **2013** 181020.
25. Caplan AI. Mesenchymal stem cells: time to change the name! *Stem Cells Transl Med* 2017; **6**: 1445–1451.
26. Roura S, Gálvez-Montón C, Mirabel C, Vives J, Bayes-Genis A. Mesenchymal stem cells for cardiac repair: are the actors ready for the clinical scenario? *Stem Cell Res Ther* 2017; **8**: 238.
27. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663–676.
28. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861–872.

29. Christoforou N, Liau B, Chakraborty S, Chellapan M, Bursac N, Leong KW. Induced pluripotent stem cell-derived cardiac progenitors differentiate to cardiomyocytes and form biosynthetic tissues. *PLoS One* 2013; **8**: e65963.
30. Singh VK, Kalsan M, Kumar N, Saini A, Chandra R. Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. *Front Cell Dev Biol* 2015; **3**: 2.
31. Mandai M, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, Fujihara M, Akimaru H, Sakai N, Shibata Y, Terada M, Nomiya Y, Tanishima S, Nakamura M, Kamao H, Sugita S, Onishi A, Ito T, Fujita K, Kawamata S, Go MJ, Shinohara C, Hata KI, Sawada M, Yamamoto M, Ohta S, Ohara Y, Yoshida K, Kuwahara J, Kitano Y, Amano N, Umekage M, Kitaoka F, Tanaka A, Okada C, Takasu N, Ogawa S, Yamanaka S, Takahashi M. Autologous induced stem-cell-derived retinal cells for macular degeneration. *N Engl J Med* 2017; **376**: 1038–1046.
32. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. *J Transl Med* 2011; **9**: 29.
33. Sugita S, Iwasaki Y, Makabe K, Kimura T, Futagami T, Suegami S, Takahashi M. Lack of T cell response to iPSC-derived retinal pigment epithelial cells from HLA homozygous donors. *Stem Cell Reports* 2016; **7**: 619–634.
34. 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**: 1313–1320.
35. Schipper RF, Schreuder GM, D'Amaro J, Oudshoorn M. HLA gene and haplotype frequencies in Dutch blood donors. *Tissue Antigens* 1996; **48**: 562–574.
36. Haimila K, Peräsaari J, Linjama T, Koskela S, Saarinenl T, Lauronen J, Auvinen MK, Jaatinen T. HLA antigen, allele and haplotype frequencies and their use in virtual panel reactive antigen calculations in the Finnish population. *Tissue Antigens* 2013; **81**: 35–43.
37. Gourraud PA, Gilson L, Girard M, Peschanski M. The role of human leukocyte antigen matching in the development of multiethnic "haplobank" of induced pluripotent stem cell lines. *Stem Cells* 2012; **30**: 180–186.
38. Taylor CJ, Bolton EM, Pocock S, Sharples LD, Pedersen RA, Bradley JA. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *The Lancet* 2005; **366**: 2019–2025.