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HLA concordance between hematopoietic stem cell transplantation patients and umbilical cord blood units: Implications for cord blood banking in admixed populations

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ABSTRACT

Umbilical cord blood stem cell transplantation is an important choice for treating a variety of hematopoietic, neoplastic, and genetic disorders. The optimal size for a cord blood bank to provide matching units for 80% of patients requiring a stem cell transplantation procedure depends on the particular characteristics of each population. In this study, we analyzed the immunogenetic diversity of a sample set of Mexican patients suffering from blood, hematopoietic, and immunological diseases, to assess the best strategy for cord blood banking. For achieving that, we analyzed *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* genotype and allele frequencies of both units from the bioarchive of the Umbilical Cord Blood Bank from La Raza and patients requiring a stem cell transplant and compared these variables with data from the same geographic and genetic context. We were able to detect significant differences for at least half of the alleles were observed for HLA class I and class II genes between units and patients. Five Native American haplotypes had lower frequencies in patients sample than in the cord blood units. Genetic admixture estimations for both groups showed a higher contribution of Native American component in the cord blood units. Differences in ancestral components in the Umbilical Cord Blood Bank from La Raza and six virtual banks modeled from a pool of Mexican mixed ancestry individuals show that genetic background is important in cord blood collection. In conclusion, increasing diversity over quantity of new cord blood units will improve the cost effectiveness of cord blood banking and health policies regarding hematopoietic stem cell transplantation in admixed populations such as those present in Latin American countries.

1. Introduction

Leukemia is the sixth leading cause of mortality in children under the age of 6 years and the second in children aged between 6 and 14 years in Mexico [1]. In 2011, the National Institute of Statistics and Geography (INEGI) of Mexico reported 4525 cases [2] of blood, hematopoietic, and immunological diseases (CIE-10 D50-D89). Some of these diseases may be treated with hematopoietic stem cell (HSC) transplantation. HSCs are immature, undifferentiated cells that are capable of indefinitely perpetuating themselves through self-renewal and of becoming any mature, differentiated cell in a sustained way [3,4]. HSCs were the first tissue-specific stem cells isolated and the first

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to be used in cellular therapy with an actual clinical benefit [5]. Umbilical cord blood (UCB) is relatively easy to collect, poses no risk to donors, and has a low likelihood of transmitting infectious diseases [6–8]. Although usually obtained in a small volume (~100 mL), HSCs obtained from this source have a high proliferative potential and, because of their naïve nature, a low risk of inducing graft-versus-host disease (GVHD). These characteristics have made UCB transplantation an important choice for treating a variety of hematopoietic, neoplastic, and genetic disorders including inborn errors of metabolism, immunodeficiencies, and leukemias [3,9,10] when no HLA-matched siblings or unrelated donors are available for HSCs transplantation. In the year 2005, cord blood gained increased attention as public bodies worldwide decided to support cord blood banking financially, in order to increase the relevant national UCB inventories [11]. Worldwide, UCB has become the second most important source of blood stem cells after PBSCs and before bone marrow [12].

There are four major public cord blood banks (CBBs) in Mexico: Centro Nacional de la Transfusión Sanguínea (CNTS); Umbilical Cord Blood Bank (UCBB) La Raza (UCBBLR), Mexican Institute of Social Security (IMSS); Mexican Bone Marrow Donor Registry (MBMDR), Instituto Nacional de Diagnóstico y Referencia Epidemiológica (INDRE); and Hospital Universitario de la Universidad Autónoma de Nuevo León [13,14]. The latter is the only one located outside Mexico City.

UCB units in UCBBLR are selected based on the total count of nucleated cells, $CD34^+$ count, viability, and HLA matching [optimal 6/6 match for *HLA-A*, *-B* (antigen-level) and *-DRB1* (allele-level); matching levels of 4/6 and 5/6 are also accepted with one restriction: having only one mismatch for *HLA-DRB1* locus [15,16].

The optimal size for a CBB to provide at least one matching unit for 80% of patients depends on the particular characteristics of each population. The numbers reported range from 1705 units in Finland, whose population is relatively genetically homogeneous, to 50,000 units in the heterogeneous UK [17]. Polymorphism is specific to a population, but despite the wide diversity observed in demographic studies, only a portion of HLA alleles and haplotypes occur in reasonable frequencies because of the influences of natural selection [18], genetic drift [19], and environmental interactions [20]. Several authors [17,21–26] have noticed that the proportions of specific ancestries impact in the number of units, both for marrow and UCB units, required for a given patient population. In Mexico, however, no studies have been done regarding the optimal size for a CBB, leading to speculation: some authors claim that 2000 units would be enough to support HSC transplantation centers in the country [27], while others claim that such diversity could only be represented in banks as large as 100,000 units [21]. In order to evaluate and improve the efficiency of UCBBLR and any other bank trying to achieve such representation of HLA genetic diversity in Mexican populations, characterization of the diversity present in the bank and in Mexican mixed ancestry populations is needed to calculate the ideal number of UCB units, taking into account size and HLA diversity.

In the present work we analyzed allelic *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* frequencies and haplotypes of the CBUs obtained by UCBBLR and compared these variables with previously published data from the same geographic and genetic context (Mexico City's metropolitan area, MCMA). Our first aim was to determine an optimal size for UCBBLR. Nevertheless, to assess whether it is not a matter of size only, but of HLA haplotypic diversity, our second goal was to closely examine the HLA heterogeneity of virtual CBUs banks using statistical tools taken from forensic and population genetics studies. We discuss the importance of HLA genetic diversity –within and between populations- in the optimization of the resources allocated for that purpose.

2. Material and methods

2.1. CBUs and patients (further details in supplementary information: patients characteristics)

UCB was collected from 521 eutocic deliveries of clinically healthy products. All mothers were Mexican Mestizo living in the northern part of Mexico City's metropolitan area. Collection of units and transplantation were performed according to the requisites of the Helsinki Declaration (2008) and the General Health Law of Mexico. All 322 patients (66% ♂; 34% ♀; mean age: 17 ± 14 years) were Mexicans by birth and have permanent residence within the country. All subjects or their legal representatives were informed about the objectives and methods used and signed an informed consent form. Only patients being born in Mexico and with at least two generations being born within the country were considered for this study [28].

Units had a total nucleated cell count of 27.3–185 ($\bar{x} = 77.7 \times 10^7$), $CD34^+$ count of 2.7–212 ($\bar{x} = 41.62 \times 10^5$), and cell viability of 85–99.83% ($\bar{x} = 95.2\%$). These values are consistent with international standards [29].

2.2. HLA typing (further details in supplementary information: HLA typing)

HLA genotyping was performed using commercially available PCR sequence-specific primers (PCR-SSP) kits (*AB/DR/DQ SSP Unitray®*, Life Technologies/Thermo Fisher Scientific Inc., Waltham, MA, USA) under ASHI requirements [30]. The resolution of HLA typings was kept as informative as possible; thus, the serological split level was used for allelic groups such as B*14 (B64, B65), B*15 (B62, B63, etc.), B*40 (B60, B61, B4005, etc.), DRB1*03 (DR17, DR18), because this information was available for most of the units and it is more informative than antigen level resolution. All alleles were classified accordingly to the WHO Nomenclature Committee for Factors of the HLA System [31]. In compliance with ASHI requirements [30], whenever a HLA typing remained unsolved after studying the complete family, or when a homozygous typing was obtained, sequence-based typing was performed to correctly assign the allele. This was the case for a total of 32 HLA typings. No new alleles were detected.

2.3. Statistical analysis (further details in supplementary information: statistical analysis)

HLA haplotypes at allelic group resolution from both UCBBLR and patients were obtained by family segregation analysis. Maximum-likelihood haplotype frequencies for alleles, two-point, three-point and four-point associations were estimated using an Expectation-Maximization (EM) algorithm provided by the computer program *Arlequin* ver. 3.5; as well as linkage disequilibrium (LD; Δ and Δ′), observed heterozygosity and expected heterozygosity and Hardy–Weinberg equilibrium (HWE). Fisher exact tests ($p = 0.05$) were made between the allele and haplotype frequencies to assess whether the distributions of HLA alleles and associations were different in both groups. Haplotypes of Native American, African, Asian, and European most-probable ancestry (MPA) were assigned based on previous reported frequencies [32–35].

Even though we use the UCBBLR data to draw a general picture on the donor-recipient matching panorama for mixed ancestry populations, this does not necessarily implies that only CBUs can be modeled this way. Any kind of approach involving collection of potential donors of HSCs would benefit from incorporating the methods described here to improve their collection strategies. Simulations to estimate the size of the bank that would satisfy 80% of the searches for compatible CBUs (6/8, 7/8 and 8/8 matching alleles for *HLA-A*, *-B*, *-DRB1* and *-DQB1*) were performed with a bootstrap resampling method [17], with 4000 bootstrap steps for each round with a script programmed in *Python™* 3.6.0 (Wilmington, USA). A percentage of 80% was decided based on

previous experiences from other authors [17,36]. For every simulation round, a patient G_i from the patients group of size $N = 322$ and a simulated CBU bank C_i of size n_i are drawn. Simulated banks were drawn from a set of Mexican mixed ancestry individuals [37,38] with phased haplotypes and equivalent resolution HLA typing from all 32 states of Mexico. To assess the role of the size and diversity of the bank in finding a match for patients, six different simulated banks with a fixed size of 6000 individuals were modeled: one with HLA typings from individuals living in the Mexico City Metropolitan Area (MCMA; including the State of Mexico) to model what would be expected if the bank would only focus on collecting CBU based on the current strategy; a second one to simulate the incorporation of the second (Guadalajara) and third (Monterrey) largest metropolitan areas assuming that efforts in increasing bank's diversity would be restricted only to similar urban areas. To construct the third, fourth and fifth virtual banks a different approach was used: we obtained three discernible regions for the whole country (Supplementary information: F_{ST} differentiation) confirmed by the exact test of sample differentiation based on haplotype frequencies [39,40]: North-western Mexico, Central Mexico and South-East Mexico. A pooled sample for each of these regions served as a new source for modeling the next three virtual banks. Lastly, a group from a randomly selected subsample from the entire data [37] set was obtained to model the whole country's diversity. All virtual banks were found to be in HWE (data not shown). For each resampling round the probability of finding a 6/8, 7/8 and 8/8 match is computed. Afterwards, Morgan-Mercer-Flodin (MMF) model equations [41] were determined for each curve and solved for x when $y = 80\%$ to determine the approximate size for each matching level (6/8, 7/8 and 8/8 matching alleles; further details in Supplementary Information: Determination of bank sizes for each matching group). To assess genetic diversity [28] of UCBBLR and patient samples, polymorphism information content (PIC) and power of discrimination (PD) were calculated [42–44] using the *PowerStat* ver. 1.2 spreadsheet (Promega Corporation, Fitchburg, WI, USA). A PIC > 0.5 is considered to be characteristic of highly polymorphic systems, and a PD value > 0.8 indicates high polymorphism in a specific population context. To analyze the ancestral genetic composition of UCBBLR and Mexican patients requiring an HSC transplant, admixture estimates were obtained using the ML method and *LEADMIX* software [45], with $k = 3$ parental populations (African, Native American, and European) and *HLA-B* allelic frequencies as genetic estimator. Finally, linear combinations were obtained from a matrix of 94 populations including both groups analyzed in this work; four subsets of a batch of CBUs from the UK [46]: European, non-European, African, and Asian; and cord blood units from the Tzu Chi Taiwan Cord Blood Bank [47]. The remaining data include 16 Native American groups, 19 Asian populations, ten African human groups, and 29 European populations (please refer to Supplementary Table 1 for further information). We also included samples from American admixed populations for comparison.

To demonstrate statistically that both the patient sample and the UCBBLR sample were different from each other and from other samples from Mexican admixed populations, the exact test of sample differentiation based on haplotype frequencies provided by the *Arlequin* software ver. 3.5 [39,40,48] was performed with a significance level of 0.05.

3. Results

Table 1 shows the total allelic variation for the *HLA-A*, *-B*, *-DRB1*, and *-DQB1* genes found for both groups. Statistically significant differences ($p < 0.05$) were found for nine of 18 *HLA-A* alleles (50%), 10 of 43 *HLA-B* alleles (23%), eight of 14 different *HLA-DRB1* alleles (57%), and most (71%) of *HLA-DQB1* alleles. It is noteworthy that HLA-B*40:02:01G may be overrepresented in the patients group as it has been associated with ALL [49]; an increase in B*40 allelic frequency in "Hispanic" children with ALL when compared to controls (14.5% versus 9.84%, p = 0.003, OR = 1.67) was found in a sample from the National Center for Blood Transfusion in Mexico City. In our ALL sample, we found that 22.1% of children with ALL carried the B*40 allele (including two B*40 homozygous patients). Table 2 and Supplementary Tables 2–4 summarize the haplotype diversity of both CBBLR and the HSC transplant patients. The frequencies of the most frequent *HLA-A ~ B ~ DRB1 ~ DQB1* haplotypes did not differ significantly between UCBBLR and the Mexican patient group; this could be due to overlapping in the ancestral parental populations of both the patients group and the UCBBLR, or a relatively small sample size. Four haplotypes (HLA-A*02 ~ B*35 ~ DRB1*08 ~ DQB1*04, A*02 ~ B*35 ~ DRB1*04 ~ DQB1*03:02:01G, A*02 ~ B*15:01:01G ~ DRB1*08 ~ DQB1*04 and A*24 ~ B*35 ~ DRB1*04 ~ DQB1*03:02:01G) were roughly twice as frequent in the former as in the latter group (Fig. 1), and all of them are commonly found in Native American populations [50–53]. The distribution of these haplotypes among Mexican populations vary considerably [38,54,55], probably because they may come from different Native American backgrounds, which are not represented homogeneously neither in mixed ancestry or Native American Mexicans [56]. Notably, the diversity exhibited by the patients group included alleles commonly found in European, Asian, and African populations. By contrast, Native American alleles were overrepresented in UCBBLR. Recent work on similar samples share parallel findings [57–59]. Those differences are noticeable in the PCA (upper left quadrant of Fig. 2), which shows that UCBBLR is more closely related to Native American groups than is to the patient sample because of differences in the *HLA-B* and *HLA-DRB1* frequencies. Other CBBs analyzed in this work showed distinct clustering tendencies. In our PCA (Fig. 2), the European CBUs in the paper of Brown et al. [46], which were collected from two London hospitals, visibly overlapped with the UK population sample from Pingel et al. [60]. The non-European units (mainly from India and Saudi Arabia) appeared between the Indian and Arab samples in a loose cluster outside the main European group. However, the African and African-descendant CBUs were located closer to the main European cluster than to the sub-Saharan African populations set. Interestingly, the "African American" category also falls outside the main sub-Saharan African cluster, which may be indicative of the European genetic component present in African Americans, as well as the influence of cultural and social conceptions.

HLA-A ~ B associations resembled the distribution observed in these two genes when studied at the allele level (Supplementary Table 2). As shown in the graphical map (Fig. 1), five haplotypes with frequencies > 2.0% were present in both samples, and several other haplotypes were shared in both groups. The *HLA-A ~ B ~ DRB1 ~ DQB1* haplotype frequencies did not differ significantly between the two groups (Fig. 1). Remarkably, the combination of three *HLA-B* alleles (HLA-B*35, -B*39, and -B*40:02:01G) and four *HLA-DRB1* variants (HLA-DRB1*04, -DRB1*08, DRB1*14, and -DRB1*16) accounted for most of the observed shared haplotypes, regardless of the *HLA-A* allele carried by the haplotype. Other extended blocks found in both samples included characteristic European (Mediterranean) haplotypes such as HLA-A*01 ~ B*08 ~ DRB1*03:01, HLA-A*03 ~ B*07 ~ DRB1*15, HLA-A*25 ~ B*18 ~ DRB1*15, and HLA-A*29 ~ B*44 ~ DRB1*07, and their *HLA-DQB1*-associated alleles [33]. More European or African associations were found in the patient group than in UCBBLR. Six blocks commonly found in Native Americans (HLA-A*02 ~ B*15:01:01G, HLA-A*02 ~ B*35, HLA-A*02 ~ B*40:05, HLA-A*02 ~ B*48, HLA-A*02 ~ B*51, and HLA-A*24 ~ B*35) had greater frequencies in UCBBLR compared with the patient group. HLA-A*24 ~ B*51 block could not be found in UCBBLR but appeared with a frequency of 0.0124 in the patient sample. This may indicate an underlying genetic structure within the Native American component and differences in the Native American contributions to both samples.

Non-Native American *HLA-B ~ DRB1* blocks were also found in higher frequencies in patients compared with UCBBLR, which in turn exhibited a higher percentage of Native American *HLA-B ~ DRB1*

Table 1
Allelic frequencies of *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* in the UCBB La Raza and the Mexican patients requiring a HSC transplant.

HLA-A					*HLA-B*					*HLA-DRB1*					*HLA-DQB1*				
	UCBBLR		Patients			UCBBLR		Patients			UCBBLR		Patients			UCBBLR		Patients	
Allele	A.F.	n (N=521)	A.F.	n (N=521)	Allele	A.F.	n (N=521)	A.F.	n (N=521)	Allele	A.F.	n (N=521)	A.F.	n (N=521)	Allele	A.F.	n (N=521)	A.F.	n (N=521)
A*01	0.0134	14	0.0373	24	B*07	0.0317	33	0.0575	37	DRB1*01	0.0355	37	0.0435	28	DQB1*02	0.0384	40	0.0792	51
A*02	0.5691	593	0.3789	244	B*08	0.0106	11	0.0171	11	DRB1*01:03	0.0019	2	0.0047	3	DQB1*03:01:01G	0.2044	213	0.2298	148
A*03	0.0259	27	0.0326	21	B*13	0.0019	2	0.0109	7	DRB1*03:01:01G	0.0173	18	0.0326	21	DQB1*03:02:01G	0.4088	426	0.3152	203
A*11	0.0115	12	0.0280	18	B*14:01	0.0029	3	0.0062	4	DRB1*04	0.4117	429	0.3214	207	DQB1*03:03:02G	0.0048	5	0.0140	9
A*23	0.0038	4	0.0186	12	B*14:02	0.0240	25	0.0404	26	DRB1*07	0.0211	22	0.0450	29	DQB1*04	0.2399	250	0.1677	108
A*24	0.1459	152	0.1429	92	B*15:01:01G	0.0864	90	0.0590	38	DRB1*08	0.2399	250	0.1724	111	DQB1*05	0.0489	51	0.0839	54
A*25	0.0019	2	0.0062	4	B*15:03:01G	0.0067	7	0.0062	4	DRB1*09	0.0010	1	0.0078	5	DQB1*06	0.0547	57	0.1102	71
A*26	0.0058	6	0.0280	18	B*15:05	0.0029	3	0.0047	3	DRB1*10	0.0029	3	0.0062	4					
A*29	0.0048	5	0.0280	18	B*15:07:01G	0.0010	1	0.0000	0	DRB1*11	0.0106	11	0.0528	34					
A*30	0.0106	11	0.0435	28	B*15:10	0.0010	1	0.0047	3	DRB1*12	0.0048	5	0.0047	3					
A*31	0.0480	50	0.0575	37	B*15:11:01G	0.0000	0	0.0031	2	DRB1*13	0.0230	24	0.0590	38					
A*32	0.0019	2	0.0140	9	B*15:15	0.0058	6	0.0016	1	DRB1*14	0.1257	131	0.1149	74					
A*33	0.0048	5	0.0233	15	B*15:16	0.0000	0	0.0031	2	DRB1*15	0.0422	44	0.0776	50					
A*36	0.0019	2	0.0047	3	B*15:17:01G	0.0010	1	0.0093	6	DRB1*16	0.0624	65	0.0575	37					
A*66	0.0010	1	0.0000	0	B*15:30	0.0019	2	0.0000	0										
A*68	0.1497	156	0.1522	98	B*15:48	0.0000	0	0.0016	1										
A*69	0.0000	0	0.0016	1	B*18	0.0038	4	0.0264	17										
A*74	0.0000	0	0.0031	2	B*27	0.0038	4	0.0155	10										
					B*35	0.2879	300	0.1693	109										
					B*37	0.0000	0	0.0047	3										
					B*38	0.0029	3	0.0186	12										
					B*39	0.1939	202	0.1724	111										
					B*40:01:01G	0.0115	12	0.0031	2										
					B*40:02:01G	0.0681	71	0.0714	46										
					B*40:05	0.0259	27	0.0109	7										
					B*40:08	0.0106	11	0.0047	3										
					B*41	0.0019	2	0.0140	9										
					B*42	0.0000	0	0.0047	3										
					B*44	0.0336	35	0.0466	30										
					B*45	0.0048	5	0.0140	9										
					B*48	0.0595	62	0.0388	25										
					B*49	0.0144	15	0.0171	11										
					B*50	0.0029	3	0.0062	4										
					B*51	0.0441	46	0.0450	29										
					B*52	0.0384	40	0.0497	32										
					B*53	0.0058	6	0.0093	6										
					B*55	0.0000	0	0.0047	3										
					B*56	0.0010	1	0.0031	2										
					B*57	0.0058	6	0.0124	8										
					B*58	0.0019	2	0.0016	1										
					B*73	0.0000	0	0.0031	2										
					B*78	0.0000	0	0.0047	3										
					B*81	0.0000	0	0.0031	2										

A.F.: Allelic frequency. Shaded cells indicate statistically different frequencies.

Table 2
HLA-A ~ B ~ DRB1 ~ DQB1 haplotypic frequencies in the UCBB La Raza sample and in the group of Mexican patients requiring a HSC transplant.

Haplotype	H.F.	n (N = 521)	Δ'	*p*	*t*	Haplotype	H.F.	n (N = 322)	Δ'	*p*	*t*
A*02 B*39 DRB1*04 DQB1*03:02:01G	0.0787	82	0.5491	0.0000	11.66	A*02 B*39 DRB1*04 DQB1*03:02:01G	0.0668	43	0.5881	0.0000	10.43
A*02 B*35 DRB1*08 DQB1*04	0.0470	49	0.1333	0.0019	4.81	A*02 B*40:02:01G DRB1*04 DQB1*03:02:01G	0.0280	18	0.5930	0.0000	6.51
A*02 B*35 DRB1*04 DQB1*03:02:01G	0.0441	46	-0.2185	0.0191	-3.28	A*02 B*35 DRB1*08 DQB1*04	0.0233	15	0.3017	0.0000	5.50
A*02 B*15:01:01G DRB1*04 DQB1*03:02:01G	0.0393	41	0.1903	0.0333	3.21	A*02 B*35 DRB1*04 DQB1*03:02:01G	0.0217	14	0.1116	0.3062	1.62
A*02 B*15:01:01G DRB1*08 DQB1*04	0.0298	31	0.2046	0.0007	5.16	A*02 B*15:01:01G DRB1*08 DQB1*04	0.0202	13	0.4254	0.0000	5.85
A*24 B*35 DRB1*04 DQB1*03:02:01G	0.0278	29	0.0345	0.7215	0.53	A*24 B*35 DRB1*04 DQB1*03:02:01G	0.0186	12	0.1924	0.1295	2.37
A*02 B*35 DRB1*14 DQB1*03:01:01G	0.0269	28	0.0986	0.0035	4.47	A*02 B*52 DRB1*14 DQB1*03:01:01G	0.0155	10	0.6833	0.0000	6.15
A*68 B*39 DRB1*04 DQB1*03:02:01G	0.0259	27	0.3496	0.0042	4.44	A*02 B*07 DRB1*15 DQB1*06	0.0155	10	0.7515	0.0000	6.29
A*02 B*40:02:01G DRB1*04 DQB1*03:02:01G	0.0259	27	0.5450	0.0000	6.42	A*68 B*39 DRB1*04 DQB1*03:02:01G	0.0155	10	0.1520	0.2599	1.78
A*02 B*51 DRB1*08 DQB1*04	0.0173	18	0.3110	0.0004	5.08	A*31 B*35 DRB1*08 DQB1*04	0.0140	9	0.6317	0.0000	5.47
A*24 B*35 DRB1*08 DQB1*04	0.0163	17	0.0182	0.7799	0.47	A*24 B*39 DRB1*14 DQB1*03:01:01G	0.0124	8	0.4132	0.0000	5.01
A*68 B*35 DRB1*08 DQB1*04	0.0163	17	0.3091	0.0007	4.92	A*33 B*14:02 DRB1*01 DQB1*05	0.0109	7	0.7677	0.0000	5.29
A*68 B*35 DRB1*04 DQB1*03:02:01G	0.0144	15	0.0180	0.8940	0.20	A*68 B*40:02:01G DRB1*04 DQB1*03:02:01G	0.0109	7	0.4714	0.0192	3.47
A*02 B*39 DRB1*14 DQB1*03:01:01G	0.0134	14	0.0057	0.8621	0.30	A*02 B*39 DRB1*14 DQB1*03:01:01G	0.0109	7	0.0209	0.6060	0.88
A*24 B*39 DRB1*14 DQB1*03:01:01G	0.0134	14	0.2823	0.0000	5.70						
A*02 B*51 DRB1*04 DQB1*03:02:01G	0.0125	13	-0.1573	0.4142	-1.23						
A*31 B*35 DRB1*08 DQB1*04	0.0125	13	0.2228	0.0213	3.48						
A*02 B*48 DRB1*08 DQB1*04	0.0115	12	0.1273	0.1620	2.22						
A*24 B*39 DRB1*08 DQB1*04	0.0115	12	0.1043	0.2385	1.90						
A*02 B*35 DRB1*16 DQB1*03:01:01G	0.0106	11	0.0444	0.3694	1.50						
A*02 B*48 DRB1*14 DQB1*03:01:01G	0.0106	11	0.2109	0.0005	4.48						
A*03 B*07 DRB1*15 DQB1*06	0.0106	11	0.8394	0.0000	6.66						
A*24 B*35 DRB1*14 DQB1*03:01:01G	0.0106	11	0.0475	0.2725	1.80						
A*24 B*35 DRB1*16 DQB1*03:01:01G	0.0106	11	0.1200	0.0002	4.57						

Only haplotypes with H.F. > 0.01 were taken into account. H.F.: Haplotypic frequency. Δ': Standardized linkage disequilibrium. *p*: Chi-square p values. *p* values < 0.05 are considered statistically significant. *t*: validation statistic parameter for two-point associations. *t* values > 2 were taken as significant. Native American MPA haplotypes are highlighted in purple, and European ones are colored in green.

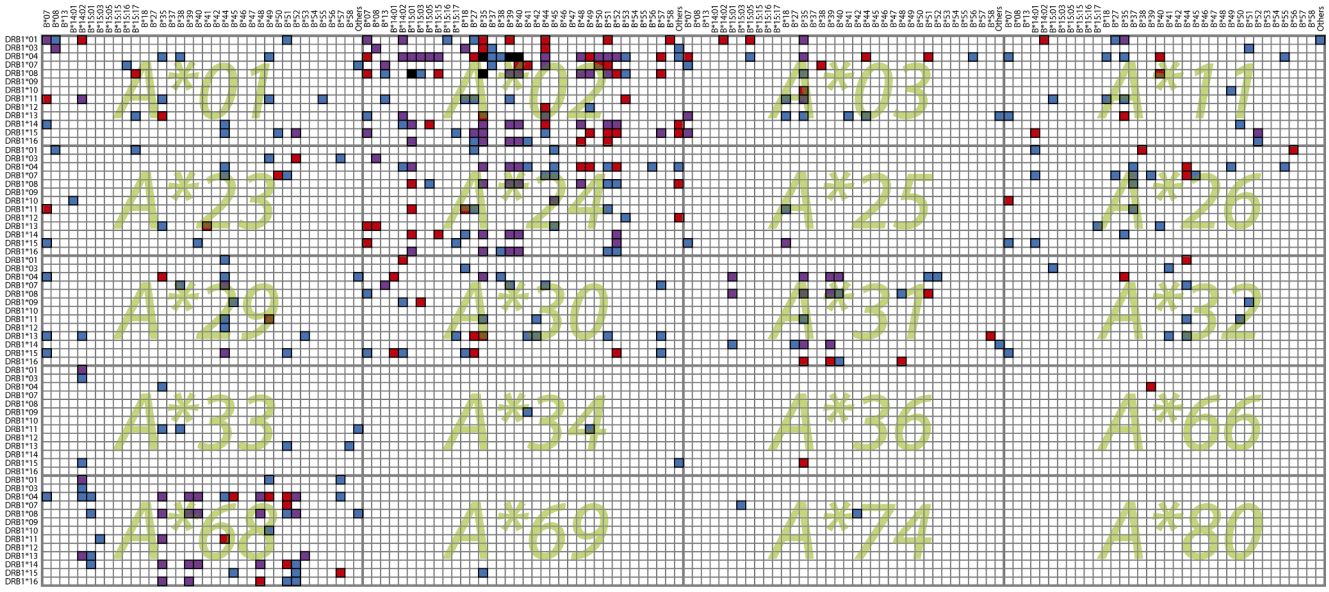

Fig. 1. Graphical map of HLA haplotype diversity compared between the UCBB La Raza and Mexican patient samples. Squares in the map result from the intersection of every possible allele for HLA-B and HLA-DRB1. Thick divisions group the combinations according to the presence of every HLA-A allele. Inside each HLA-A rectangle, HLA-B alleles are organized in the horizontal direction in each quadrangle, and HLA-DRB1 alleles are ordered vertically. Red squares represent those haplotypes found in the UCBB La Raza sample but not in the patient sample, blue squares indicate haplotypes found in the patient but not in the UCBB La Raza sample, and purple squares show haplotypes present in both groups. Black squares show the presence of HLA-A/-B/-DRB1 combinations present in both samples in frequencies > 2.0%.

associations (Supplementary Table 3). The HLA class II blocks mirror the trends in the frequencies and distributions of the *HLA-DRB1* gene within the analyzed samples, which would be expected in a system with a high LD (Supplementary Table 4). Only five haplotypes commonly found in Native American populations (HLA-A*02 ~ B15:01 ~ DRB1*04 ~ DQB1*03:02:01G, HLA-A*02 ~ B*35 ~ DRB1*04 ~ DQB1*03:02:01G, HLA-A*02 ~ B*35 ~ DRB1*08 ~ DQB1*04, HLA-A*02 ~ B*35 ~ DRB1*14 ~ DQB1*03:01:01G, and HLA-A*02 ~ B*39 ~ DRB1*08 ~ DQB1*04) had higher frequencies in UCBBLR than in the patient sample (Table 2). The rest of the haplotypes with significant frequencies did not differ significantly regardless of their proposed origin.

The exact test of sample differentiation based on haplotype frequencies showed that all admixed populations included differed statistically from each other ($p < 0.0001$) in terms of the HLA system at the haplotype level. Results for HLA genetic diversity and genetic composition in both samples and the six virtual banks are listed in Table 3. Except for the South East Mexico bank, all virtual banks were shown to be at least as diverse as the patients sample when PIC and PD were taken into account. The results from the bootstrapping method for determining the size of the CBU banks number of CBUs necessary to provide 80% of the patients with a compatible unit for each matching level are summarized in Supplementary Table 5. Since few banks would actually offer at least 80% of the patients with a compatible donor, equations were used to model bank sizes up to 100,000 units and assess, with such banks, what is the percentage of leukemia patients that could get a matched donor. At 100,000 units collected, 40.30% of patients would have at least one matching option (8/8 alleles) with a whole-country approach, whereas nearly ¼ of all patients would get a matching donor if banking is restricted to Central Mexico. Interestingly, the whole country has an estimated 13.69% of African ancestry, the highest estimation for a virtual bank. Whole Mexico virtual bank also ranks 3rd regarding European ancestry and is in the second-to-last position in Native American ancestry (54.80%, still above the 45.74% of Native American contribution estimated for the patients). Coefficients for the equations as well as correlation coefficients for each bank size simulation were included in Supplementary Information: Determination of bank sizes for each matching group.

4. Discussion

4.1. Allelic diversity in the HLA system in patients and cord units.

Both the UCBBLR and the patients' sample sets, as well as Latin American admixed populations including a Mexican American sample and a sample of the "Hispanic" population in the USA included in this analysis, could be differentiated by their *HLA-B* and *HLA-DRB1* frequencies. This implies that genetic admixture analysis should play an important role in the improvement and development of CBBs and search algorithms for a suitable donor–recipient coupling. Populations regarded as "Mexican," such as Puebla and Guadalajara, are clearly distinguishable from each other and may be considered as independent populations with characteristic genetic pools. Wide categories such as "Hispanic" minimize the genetic diversity of very different populations and simplify them within an imprecise term that does not represent the actual heterogeneity of Latin American human groups. For instance, genetic admixture estimations for UCBBLR, the six virtual banks and patients' samples were shown to be different. However, the best strategy (banking UCB from the whole country) differed considerably from the other approaches. This means that it is not a matter of number of units, or diversity itself, but also the amount of each biological root's proportion that plays a major role in selecting the best banking strategy. Previous reports [54,55] have shown differences in the admixture proportions throughout Mexico, especially regarding African biological roots. This uneven African contribution could be one of the reasons why a whole-country strategy would be significantly better than regional approaches regarding banking CBU (or even bone marrow donor) units.

4.2. HLA associations and haplotypes: two completely different scenarios

Despite the notable differences in allele distribution within the samples studied, two point associations and haplotypes followed a dissimilar pattern. However, numbers for banks able to offer a match for leukemia patients dist from what realistically could be achieved.

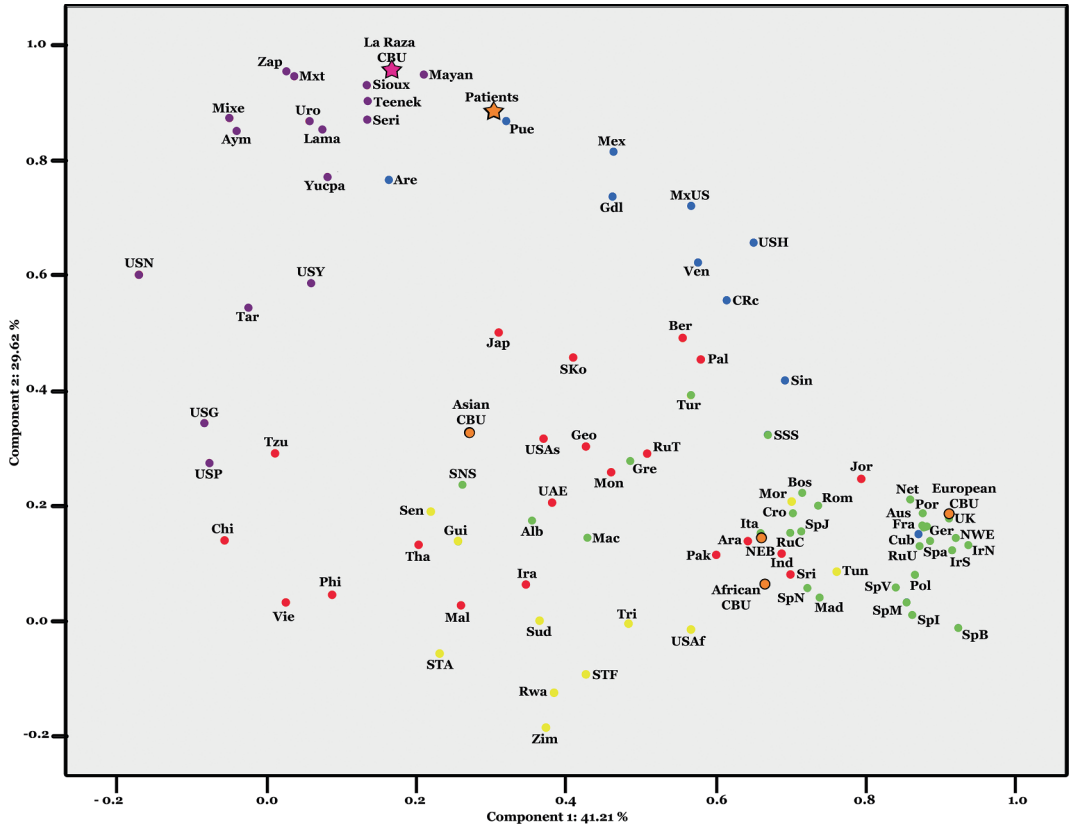

Fig. 2. Principal component analysis. European CBU, non-European CBU (NEB), African CBU, and Asian CBU, and Cord blood units from the Tzu Chi Taiwan Cord Blood Bank (Tzu) are represented by orange dots, and the La Raza CBU sample is marked with an orange star. The patients group is represented by a pink star. Native American populations are represented by purple dots: Yup'ik (USY), Pima (USP), Gila River Indian Community (USG), Sioux, Native Americans (USN), Tarahumaras (Tar), Teenek, Mixe, Mixtec (Mxt), Zapotec (Zap), Seri, Maya, Yucpa, Lama, Uro, Aymara (Aym). Asian populations are marked with red dots: Lisu (Chi), Maratha (Ind), Baloch (Ira), Japan (Jap), Jordanians (Jor), Malays (Mal), Mongolia (Mon), Palestinian (Pal), Parsi (Pak), Ivatan (Phi), Aleut (Ber), Guraiat and Hail (Ara), Tuvans (RuT), South Korea (SKo), Sinhalese (Sri), Thai (Tha), Turkey (Tur), United Arab Emirates (UAE), Kinh (Vie). Yellow dots represent African populations: Guiné-Bissau (Gui), Moroccans (Mor), Rwanda (Rwa), Angolares (STA), Forros (STF), Senegal (Sen), Sudan (Sud), Zimbabwe (Zim), Trinidad & Tobago (Tri), Tunisians (Tun), African American from USA (USAf). Green dots correspond to European human groups: Albania (Alb), Austria (Aus), Bosnia and Herzegovina (Bos), Croatia (Cro), England (NWE), France (Fra), Svans from Georgia (Geo), Germany (Ger), Greece (Gre), Ireland (IrS), Northern Ireland (IrN), Italy (Ita), Macedonians (Mac), Madeirans (Mad), Netherlands (Net), Poland (Pol), Portugal (Por), Romania (Rom), Chuvashian (RuC), Chelyabinsk Region (RuU), Spain (Spa), Arratia Valley (SpB), Ibiza (SpI); Majorca (SpJ); Minorca (SpM), Pas Valleys (SpV), Northern Swedish Sami (SNS), Southern Swedish Sami (SSS), United Kingdom (UK). Admixed populations are marked with blue dots: Costa Rica (CRc), Cuba (Cub), "Hispanics" from USA (USH), Mexican Americans (MxUS), Mexico City (Mex); Guadalajara, Mexico (Gdl); Puebla, Mexico (Pue); Sinaloa, Mexico (Sin); Arequipa, Peru (Are); Venezuela (Ven).

Whilst five different strategies (Supplementary Table 5) need a relatively low number of units at a 6/8 alleles matching level, numbers as high as 100,000 units would not suffice to provide suitable units to ~80% of the patients requiring a stem cell transplant at 7/8 and 8/8 matching levels. Other authors [36] reported that 50,000 units would be needed to provide 80% of the European ancestry patients with a 5/6 (equivalent to 7/8 in our study) HLA match level unit in the United Kingdom. As a biologically three-rooted mixed ancestry population, it is expected that Mexican patients would need a higher number of units to encompass the diversity coming from the Native American, African and European components. It is not the allelic frequency that influences the selection of donor–recipient couples, but the haplotype diversity which is tightly tied to biological ancestries. As our results show, increasing the number of units is not useful if units are not representative of the actual biological diversity of Mexican mixed ancestry populations where the patients come from. Finally, several of the haplotypes that

Table 3
HLA genetic diversity and genetic composition in both samples.

	PIC				PD				Ancestral contributions		
	HLA-A	*HLA-B*	*HLA-DRB1*	*HLA-DQB1*	*HLA-A*	*HLA-B*	*HLA-DRB1*	*HLA-DQB1*	Native American	European	African
UCBBLR	0.60	0.84	0.72	0.63	0.83	0.96	0.90	0.67	0.6937	0.2476	0.0587
All Mexico	0.80	0.90	0.83	0.78	0.95	0.99	0.96	0.94	0.5480	0.3125	0.1329
Center	0.77	0.88	0.82	0.76	0.94	0.98	0.96	0.93	0.6577	0.2373	0.0227
Main cities	0.80	0.90	0.83	0.78	0.95	0.99	0.96	0.94	0.5550	0.3409	0.0337
North	0.84	0.93	0.86	0.80	0.97	0.99	0.97	0.95	0.3969	0.4809	0.0705
South	0.82	0.86	0.76	0.72	0.95	0.97	0.93	0.91	0.7409	0.1985	0.0541
MCMA	0.78	0.90	0.82	0.77	0.94	0.98	0.96	0.93	0.6011	0.2905	0.1084
Patients	0.78	0.91	0.82	0.74	0.94	0.98	0.95	0.77	0.4574	0.4303	0.1124

PIC: Polymorphism informative content; PD: Power of discrimination; UCBBLR: Umbilical Cord Blood Bank La Raza; MCMA: Mexico City Metropolitan Area.

are found only in either patients or units corresponded to those commonly found in Asian or African human groups, as well as Native American haplotypes present in specific regions or mixed-ancestry haplotypes. Native American components are not distributed homogeneously within Mexico, and even inside regions associated with one macro linguistic family, genetic substructure can arise [56,61] as a result of distinct demographic histories for different Native American groups [62–64]. For this reason, a whole-country approach for the collection of HSC donors would be more helpful when trying to achieve a better representation of the biological roots underlying in the immunogenetic diversity seen in mixed ancestry Mexicans. As shown, it is relevant to use population-wide statistical tests (like the exact test of sample differentiation based on haplotype frequencies) to better spot differences between datasets that may be masked by less powerful, but more widely used tests which regard differences only at the frequency-level (like the Fisher test).

4.3. Implications of HLA allelic and haplotype diversity for efficient cord blood banking in admixed populations

Similarities in UCBBLR and the patients requesting a CBU may reflect the parallel demographic histories of both groups within the population context of three main biological roots (Native American, African, and European) living and procreating without any significant barriers. The biological diversity exhibited by modern Latin American populations is rooted in diverse populations including millions of Native Americans living in distinct environments [65] and with different genetic backgrounds [56]; almost half a million Europeans, mainly peninsular Spaniards, but also French, German, and English colonizers [66]; > 150,000 biologically and culturally diverse African enslaved immigrants from tens of human groups from sub-Saharan Africa [67,68]; unknown numbers of other ethnic groups and minorities such as North Africans, Middle Easterners and Romani [69]; and Asian migrants from South East Asia who arrived throughout the colonial period [70].

The resulting biological diversity can be overlooked because of the use of loose terms such as “Mestizo” or “Hispanic,” or concepts such as a “Mexican genome” or “Mexican haplotypes”. The PCA plot visually demonstrates that, even though admixed populations are distributed between the European and the Native American clusters, differences in the ancestral composition of each sample makes it impossible to group them together as a unit. Instead, the density of haplotypes and phenotypes derived from this complex context should be acknowledged. It is thus evident that collection procedures, although efficient, need to be improved by comprising wider areas, not only by geography, but also by ethnic and social traits. We propose that a professional interdisciplinary team should be developed to collect CBUs from different allocations to increase not the size, but also the diversity of the bank. Since the average nominal cost of recruitment, collection, banking, and storage for each UCB unit actually stored ranges from 1092 USD to 1830 USD [11,12,27], strategies are needed to optimize both the financial and logistic efforts needed to provide the highest number of patients with a suitable unit in the registry.

The fine details about the disparities in the frequencies of specific HLA alleles, blocks, or haplotypes reflect factors that should be considered when explaining the biological diversity observed in both groups. Possibly the most important factor is that patients requiring an HSC transplant should not be viewed as a population but as a group of Mexican individuals from all over the country. On the other hand, UCBBLR collects units from three hospitals, all of which are located within a specific geographic and social context: the northern part of the metropolitan area of Mexico City. This region has specific migration patterns [59,71], which include immigration of individuals from states with a high proportion of Native American populations such as Hidalgo (30%), Oaxaca (58%), and Puebla (25%). This explains the higher frequencies of alleles and associations of that ancestry in the CBUs from UCBBLR. And that lack of diversity in the current approach also make it evident why a whole-country strategy is more suited for better comprise the immunogenetic diversity of mixed ancestry patients. These observations, together with the fact that other “Mestizo” populations from Mexico and Latin America can be distinguished from each other by their HLA frequencies (and thus should not be categorized together), raises the consideration of two points that must be included in the scope of cord blood banking. First, diversity may be more important than quantity when providing patients with a compatible CBU [72]. Second, to provide a cost-effective CBB, special attention should be directed to collecting units from different social and geographic contexts that adequately represent the vast diversity of HLA phenotypes observed in patients, providing a more heterogeneous genetic background to HSC banking. Based on an average estimated cost of 1118–1524 USD for UCB products [12,27], using a whole-country strategy to increase the number of units in the UCBBLR, instead of focusing only in the main cities or keeping the current strategy (MCMA) could save between 517,600–705,600 USD and 1,308,000–1,783,000 USD, respectively. For a completely matched unit, sampling 100,000 units from the whole country would provide only ≈40% of patients with a suitable donor. But any other strategy would fail to provide at least 1/3 of the patients with a potential donor. If such an economic effort shall be made, the best way to proceed is trying to represent the whole diversity of Mexican mixed ancestry populations. It is of importance to note that Southeastern Mexico has a very distinctive genetic composition (Supplementary Information: F_{ST} differentiation) that could be related with admixture with Maya populations [56], and banking strategies for that region may need to be tailored to that particular genetic background.

The understanding of population-level statistical tools to analyze the biological diversity of a population should be incorporated into the strategies for developing and improving CBBs and marrow donor lists. For example, the first step towards generating a successful bank is to collect sufficient units so PD and PIC values are above those shown by the specific patient group, thus exceeding the diversity demanded by the patients’ genetic background. This is the case for all modeled banks except for Southern Mexico. UCBBLR has lower diversity values than the patients group and that any simulated bank. However, it is not just a matter of allelic diversity, because then one would expect that all banks exceeding PIC or PD values of those in the patients group could be enough to provide patients with suitable donors, which is not the case. It is also a matter of the biological roots represented in that diversity.

5. Conclusion

Biological diversity of populations should be acknowledged, especially in HLA-related clinical issues. Loose categories can be costly because of the difficulty in achieving specific goals regarding the representation of the biological diversity in such wide human groups. Instead, efforts should be made to represent more accurately specific subgroups. Ethnicity, ancestral genetic contribution, social and economic factors should be considered when collecting CBUs to provide more patients with compatible units. The analysis shown in this work and the interactions between distinct social and ethnic groups (including migration), should be considered not only in cord blood banking, but in any other HSC collection strategy in admixed contexts. Applying these observations will improve the understanding and representation of biological diversity of CBBs and HSC collection and the cost effectiveness of health policies regarding HSC transplantation.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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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.2019.05.002.

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