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Original Synthetic Article

HLA genes in Chimila Amerindians (Colombia), the Peopling of America and Medical implications

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Abstract – Our aim is to study the HLA-A, -B, -C, -DRB1 and -DQB1 gene frequencies in the Chimila Amerindian (Colombia) ethnic group. Results are compared with other World populations in order to obtain information about Chimila and Amerindian Health promotion, Amerindian origins and America peopling. Written consent was obtained from Chimila subjects to be included in this study. Peripheral blood was drawn and HLA DNA genotyping was carried out by standard methods. Analyses of Chimila relatedness with other Amerindians and worldwide populations was performed with a standard NJ dendrogram and correspondence analyses methodology. Chimila HLA gene profile showed to be related to that of other Amerindians groups. New complete HLA extended haplotypes were obtained. Some of them are described for the first time. Also, specific genealogical comparisons were done between Chimila Amerindians and Pacific Islanders by using specific HLA alleles. Our conclusions are: 1) These new data and HLA extended haplotypes are useful for present and future Chimila Preventive Medicine (HLA linked diseases), HLA Pharmacogenomics and transplantation regional programs, 2) Classical accepted origin of America peopling should be revised: Pacific (Asian and Austronesian) and Atlantic (European) populations gene exchange may have occurred before 1492 AD. This is confirmed by our present studies using HLA autosomic genetic markers. 3) Genetic HLA Amerindian profile is separated from that of other World populations.

Keywords: America peopling, Amerindians, Anthropology, Chimila, Epidemiology, HLA and Transplantation.

Introduction

Chimila ethnic group is living at the San Angel savannas area in the Department of Magdalena, Colombia (Figure 1). Its mother language is "*ette taara"* that belongs to the Chibchan speaking group of languages (Ruhlen 1987).

First Amerindian Native populations are postulated to have come from Asia through the Bering land bridge between 30,000–12,000 years before the present (BP). Greenberg first postulated the triple migration theory for explaining the peopling of the Americas (Greenberg et al, 1986): Amerindians (most North and South American Indians; 12,000 years BP), Na-Dene (Athabaskans, Navajo, Apache; 8,000 years BP) and Eskimo-Aleuts (6,000 years BP). However, other authors postulate only one wave coming from Mongolia / North China as giving rise to the First Native American ancestors (Kolman et al. 1996; Merriwether et al. 1996).

In addition, origin of Amerindians and other First America Inhabitants coming from Asia has been put forward through Coastal Pacific line (Goebel et al. 2008) based on all available archaeological, anthropological and mtDNA and genetic data. Alu-insertion investigations have also been carried out to ascertain the origin of First Americans (Novick et al. 1998). The results are not concordant with the multiple-wave migration hypothesis; a surprisingly short genetic distance between Chinese and Native Americans was found and explained by a recent gene flow from Asia (Novick et al. 1998). A Trans-Pacific route of American peopling from Asia or Polynesia has also been suggested because HTLV-1 virus strains shared identical sequences in Japan and in the northern coast of South America (Leon-S et al. 1996) and some HLA alleles may have been introduced by the same Trans-Pacific route (Arnaiz-Villena et al. 2009). In the same way, "quasi-specific" Amerindian HLA alleles, like A*02:12 or B*39:05 (Arnaiz-Villena et al. 2005), have been found in several unrelated individuals of Easter Island, which suggests an early contact between Easter Islanders Polynesians and Amerindians (Lie et al. 2007). Recent genetic studies have identified Polynesian mtDNA haplogroups in remains (skulls) of Botocudo Amerindians from Brazil (Amerindian group extinct by the end of 19th century) (Goncalves et al. 2013).

On the other hand, both genetic (Bruges-Armas et al. 1999) and archaeological (Holden 1999) evidence suggests that a two-way Trans-Atlantic traffic occurred before

Columbus discovered America; archaeologists in New Mexico have recently found tools used 20,000 years ago in Spanish Solutrean culture (Holden 1999; Stanford and Bradley 2012).

Indeed, we have already concluded from our previous studies on HLA genes that: 1- Aleuts seem to be a genetic and linguistic separate group which may be related to northern European Lapps, both of them originated in southern Siberia Baikal Lake area. 2- First America Inhabitants, including all analyzed Amerindians, Na-Dene speakers and Eskimo have had genetic flow with Pacific Islanders: the latter share autosomal HLA alleles and haplotypes with First America Inhabitants. This could have been bidirectional. 3- Particularly, Easter Islanders show a probable cultural and genetic exchange with Titikaka Lake Aymaras. This civilisation also shares significant traits with European Iberian megalithic builders. 4- Mesoamericans may be grouped together because of their bear more ancient Olmec culture traits and present paper HLA results. 5- Genetics is not able by itself to uncover in space and time Americas peopling and First America Inhabitants relatedness with Pacific Islanders (Arnaiz-Villena et al. 2014a).

In the present work, we have studied the Chimila ethnic group HLA allele frequencies and have compared them with those of Caucasian European, Siberians, Orientals, Na-Dene, Eskimos and Amerindian populations. HLA genes have been analyzed for the following Amerindian ethnic groups: Mayans (Gomez-Casado et al. 2003), Mixe, Mixtecans, Zapotecans (Petzl-Erler et al. 1997), Lakota Sioux (Leffell et al. 2004), Mazatecans (Arnaiz-Villena et al. 2000), Lamas (Moscoso et al. 2006), Quechuas (Martinez-Laso et al. 2006), Aymaras (Arnaiz-Villena et al. 2005), Uros (Arnaiz-Villena et al. 2009), Tarahumaras (Garcia-Ortiz et al. 2006), Mapuches (Rey et al. 2013), Toba Pilaga, Mataco Wichi, and Eastern Toba (Cerna et al. 1993). Thus, our aims are: 1) To determine the HLA class I (-A, -B and -C) and HLA class II (-DRB1 and -DQB1) quasi-specific Chimila Amerindian allelic lineages (hereafter ‘‘alleles’’ for simplicity) and specific HLA haplotypes by using a HLA DNA typing technique based on Luminex probe standard methodology; in other words, the most frequent HLA alleles and haplotypes in this ethnic group which do not exist or exist in very low frequency in other populations, i.e.: genealogy comparisons, 2) To compare the Chimila Amerindians HLA allele frequencies with those of other First American Natives (Na- Dene, Eskimo and Aleuts) and also those of Asian and Pacific populations with computer programs in order to study the HLA relatedness with peoples most likely to be candidates for First

American Peoples ancestors; this would clarify the still unclear peopling of the Americas and the origins of Amerindians, i.e.: groups of genes frequencies comparisons by using class II HLA alleles and haplotypes frequencies, 3) To studying specific HLA alleles that are very frequent to Pacific Ocean people and common to Pacific Ocean people and our Chimila sample and 4) To establish the Chimila profile that will be useful for preventive HLA genetic epidemiology (HLA linked diseases), HLA pharmacogenomics and future transplant waiting lists.

Fig. 1. Map of San Angel city zone. *Area where the Chimila ethnic group inhabits.*

Material and methods

Forty-seven healthy unrelated individuals from the Chimila ethnic group were HLA class I and class II typed. Nowadays, the Chimila group is limited to a marginal territory at the San Angel savannas in the Department of Magdalena, Colombia (Figure 1). Each individual was born in this area, had a Chimila physical appearance and their four grandparents had been born in the same area and spoke their own language, *ette taara*, belonging to Chibchan linguistic family. A written consent to participate in the present study was signed by each individual. We compare our Chimila HLA data with those of Pacific Islanders, Caucasian Europeans, Siberians, Orientals, Na-Dene Eskimos and Amerindian populations, (11th, 12th International HLA Workshop (Clayton and Lonjou. 1997; Imanishi et al. 1992a)) obtaining the genetic distances (comparison was done with 15,626 chromosomes) (Table 1), relatedness dendrograms and correspondence analysis. In particular, Amerindian populations include tribes from the following linguistics families Arawakan (Wayu and Terena Indians), Chibchan (Arsario, Kogi, Arhuaco, and Cayapa), Choco-Embera (Jaidukama), Ge Pano Caribe (Xavantes, Mataco and Toba), Mayan (Mayans), Mixe-Zoque (Mixe), Oto-Manguean (Mixtecans, Mazatecans and Zapotecans), Uru-Chipaya (Uros), Uto-Aztecan (Nahuas and Mayos) and Andean groups like Aymara, Quechuas and Lamas (Lewis et al. 2014; Ruhlen 1987; Swadesh 1959).

HLA class I (A, B and C) and high resolution HLA class II (DRB1 and DQB1) was performed by PCR-SSOP Luminex technique (Itoh et al. 2005) as referenced in (Arnaiz-Villena et al. 2014b; Arnaiz-Villena et al. 2015).

Statistical analysis was performed with Arlequin v3.0 software kindly provided by Excoffier and Slatkin (Excoffier et al. 2005). In summary, this program calculated HLA-A, -B, -C, -DRB1, and -DQB1 allele frequencies, Hardy–Weinberg equilibrium and the linkage disequilibrium between *n* alleles at *n* different loci. Their level of significance (*P*) for 2 x 2 comparisons was determined as previously described (Imanishi et al. 1992b; Imanishi et al. 1992c). In addition, the most frequent complete haplotypes were deduced from: 1) the 2, 3, 4 and 5 HLA loci haplotype frequencies (Imanishi et al. 1992b; Imanishi et al. 1992c); 2) the previously described haplotypes in other populations (Imanishi et al. 1992b; Imanishi et al. 1992c); and 3) haplotypes if they appeared in two or more individuals and the alternative haplotype was well defined (Imanishi et al. 1992b; Imanishi et al. 1992c).

Table 1. Populations studied in the present work. A total of 15,626 chromosomes were analyzed (*N* = individual numbers).

Population	*N*	Reference	Population	*N*	Reference
Chimila	47	Present Study	Buyi	70	(Imanishi et al. 1992a)
Mazatecans	89	(Arnaiz-Villena et al. 2000)	Manchu	50	(Imanishi et al. 1992a)
Mayans	132	(Gomez-Casado et al. 2003)	Koreans	100	(Imanishi et al. 1992a)
Aymara	102	(Arnaiz-Villena et al. 2005)	Japanese	493	(Imanishi et al. 1992a)
Lamas	83	(Moscoso et al. 2006)	Khalk Mongolians	202	(Munkhbat et al. 1997)
Quechuas	80	(Martinez-Laso et al. 2006)	Tuvinians	197	(Martinez-Laso et al. 2001)
Seri	100	(Petzl-Erler et al. 1997)	Khoton Mongolians	85	(Munkhbat et al. 1997)
Mixe	55	(Petzl-Erler et al. 1997)	Germans	295	(Imanishi et al. 1992a)
Mixtecans	103	(Petzl-Erler et al. 1997)	Danish	124	(Imanishi et al. 1992a)
Zapotecans	75	(Petzl-Erler et al. 1997)	Sardinians	91	(Imanishi et al. 1992a)
Mexican Mestizo	99	Unpublished Results	Albanians	65	(Imanishi et al. 1992a)
Wayu	58	(Silvera et al. 2011)	Italians	184	(Imanishi et al. 1992a)
Arhuaco	123	(Yunis et al. 1994)	French	179	(Imanishi et al. 1992a)
Kogi	67	(Yunis et al. 1994)	Spaniards	176	(Martinez-Laso et al. 1995)
Arsario	20	(Yunis et al. 1994)	Spanish Basques	80	(Martinez-Laso et al. 1995)
Cayapa	100	(Titus-Trachtenberg et al. 1994)	Algerians	102	(Arnaiz-Villena et al. 1995)
Xavantes	74	(Cerna et al. 1993)	Berbers (Souss)	98	(Izaabel et al. 1998)
Guarani	32	(Petzl-Erler et al. 1997)	Macedonians	172	(Arnaiz-Villena et al. 2001)
Toba-Pilaga	19	(Cerna et al. 1993)	Cretans	135	(Arnaiz-Villena et al. 1999)
Mataco-Wichi	49	(Cerna et al. 1993)	Ashkenazi Jews	80	(Martinez-Laso et al. 1996)
Eastern-Toba	135	(Cerna et al. 1993)	Non Ashkenazi Jews	80	(Martinez-Laso et al. 1996)
Jaiduakama	39	(Martinez-Laso et al. 2011)	Lebanese NS	59	(Clayton and Lonjou, 1997)
Tarahumara	44	(Garcia-Ortiz et al. 2006)	Lebanese KZ	93	(Clayton and Lonjou, 1997)
Nahuas	85	(Vargas-Alarcon et al. 2007)	Moroccans Jews	94	(Roitberg-Tambur et al. 1995)
Mayos	60	(Arnaiz-Villena et al. 2007)	Bushmen	77	(Imanishi et al. 1992a)
Teenek	55	(Vargas-Alarcon et al. 2006)	South African Blacks	86	(Imanishi et al. 1992a)
Terena	60	(Lazaro et al. 1999)	North American Blacks	132	(Imanishi et al. 1992a)
Uros	105	(Arnaiz-Villena et al. 2009)	Chuvashians	82	(Arnaiz-Villena et al. 2003)
Lakota Sioux	302	(Leffell et al. 2004)	Russians	200	(Kapustin et al. 1999)
Eskimos	35	(Grahovac et al. 1998)	Western Samoa	102	(Gao et al. 1992c)
Athabascans	124	(Monsalve et al. 1998)	Madang	65	(Gao et al. 1992a)
Nivkhs	32	(Grahovac et al. 1998)	Rabaul	60	(Gao et al. 1992a)
Tlinglit	53	(Imanishi et al. 1992a)	New Caledonia	65	(Gao et al. 1992a)
Udegeys	23	(Grahovac et al. 1998)	Fidji	57	(Gao et al. 1992a)
Koryaks	92	(Grahovac et al. 1998)	Papua New Guinea	57	(Gao et al. 1992a)
Chukchi	59	(Grahovac et al. 1998)	Central Desert	152	(Lester et al. 1995)
Kets	22	(Grahovac et al. 1998)	Ainu	50	(Bannai et al. 1996)
Evenks	35	(Grahovac et al. 1998)	Yuendumu	119	(Lester et al. 1995)
Aleuts	105	(Rey et al. 2010)	Cape York	80	(Gao et al. 1992b)
Singapore Chinese	71	(Imanishi et al. 1992a)	Kimberley	82	(Gao et al. 1992b)

In order to compare phenotype and haplotype HLA frequencies with other populations, the reference tables of the 11th and 12th International HLA Workshops were used (Clayton and Lonjou. 1997; Imanishi et al. 1992a) and www.allelefrequencies.net web page (Gonzalez-Galarza et al. 2015). Phylogenetic trees (dendrograms) were constructed with the allelic frequencies using the Neighbour-Joining (NJ) method (Saitou and Nei 1987) with the genetic distances between populations (DA) (Nei 1972), using DISPAN software comprising the programs GNKDST and TREEVIEW (Nei 1973; Nei et al. 1983). Correspondence analysis in three dimensions and its bidimensional representation was carried out using the VISTA v5.05 computer program (Young and Bann 1996), http:/forrest.psych.unc.edu). Correspondence analysis consists of a geometric technique that may be used for displaying a global view of the relationships among populations according to HLA (or other) allele frequencies. This methodology is based on the allelic frequency variance among populations (similar to the classical components methodology) and of a statistical visualization of the differences.

Results

Characteristic HLA allele frequencies found in Chimila population; comparisons with other World populations

The expected and observed gene frequency values for HLA-A, -B, -C, -DRB1, and -DQB1 loci do not differ significantly and the population is found in Hardy–Weinberg equilibrium (data not shown). Table 2 shows HLA allele frequencies found in our Chimila population. Twenty different HLA-A, twenty-nine different HLA-B, and fifteen different HLA-C alleles were found in Chimila sample. However, only six HLA-A alleles, four HLA-B alleles, and four HLA-C alleles, (A*02:01, A*02:04, A*24:02, A*24:03, A*29:02, A*68:01, B*35:43, B*40:09, B*51:10, B*78:02, C*01:02, C*03:04, C*07:01, and C*15:02) had frequencies higher than 4%. With regard to the HLA class II alleles, seventeen different HLA-DRB1 and thirteen different HLA-DQB1 alleles were found. Only five HLA-DRB1 and four HLA-DQB1 alleles had frequencies higher than 4% (DRB1*03:02, DRB1*04:04, DRB1*04:07, DRB1*04:17, DRB1*15:01, DQB1*03:01, DQB1*03:02, DQB1*04:02, and DQB1*06:02). Most alleles had already been found in Amerindians. DQB1 allele frequencies reflect the DRB1 locus allele distribution due to the strong linkage disequilibrium between these two loci.

Table 2. HLA-A, -B, -C, -DRB1, and -DQB1 allele frequencies in Chimila population

Allele	Allele frequencies (%)	Allele	Allele frequencies (%)	Allele	Allele frequencies (%)
	HLA-A		**HLA-B**		**HLA-DRB1**
01:01	2.1	38:01	2.1	01:01	1.1
02:01	6.4	39:01	3.2	03:01	2.1
02:04	4.3	39:04	1.1	03:02	9.6
02:46	1.1	39:05	2.1	03:07	1.1
02:58	1.1	39:09	1.1	04:04	4.3
02:87	1.1	39:13	1.1	04:07	53.2
02:93	1.1	40:09	4.3	04:17	6.4
23:01	2.1	40:89	1.1	07:01	1.1
24:02	21.3	44:03	1.1	08:04	2.1
24:03	7.5	45:01	3.2	08:07	1.1
26:01	1.1	48:01	1.1	11:02	1.1
29:02	4.3	51:10	40.4	13:01	3.2
30:01	2.1	51:24	1.1	14:06	1.1
30:02	2.1	51:46	1.1	14:07	1.1
31:01	1.1	57:01	1.1	15:01	6.4
31:08	1.1	68:01	1.1	15:03	3.2
32:01	1.1	78:02	4.3	16:02	2.1
68:01	10.6		**HLA-C**		**HLA-DQB1**
68:23	2.1	01:02	17.0	02:01	3.2
74:01	1.1	02:10	2.1	02:02	1.1
	HLA-B	03:04	11.7	03:01	7.5
07:08	1.1	04:01	1.1	03:02	54.3
08:01	3.2	04:07	1.1	03:03	3.2
08:50	2.1	06:02	3.2	03:04	1.1
14:02	1.1	07:01	7.5	03:18	1.1
15:01	2.1	07:05	1.1	04:02	10.6
15:03	1.1	07:27	1.1	04:03	1.1
15:17	1.1	08:01	1.1	05:01	1.1
15:47	2.1	08:02	1.1	06:02	10.6
35:31	1.1	12:03	2.1	06:03	3.2
35:43	13.8	15:02	46.8	06:04	2.1
35:44	1.1	16:01	1.1		
35:68	1.1	17:01	2.1		

Two types of analysis were done in order to compare Chimila HLA frequencies with other World population frequencies: 1) with pooled DRB1 and DQB1 data (not shown); and 2) with DRB1 only. It was not possible to carry out a study comparing HLA class I allele frequencies or HLA class I and II conjointly due to the lack of class I studies in many worldwide populations. The single DRB1 study was carried out in order to compare the Chimila HLA population frequencies with those of Caucasoids and Siberians who lacked DQB1 analyses. The Neighbour-Joining (NJ) relatedness dendrogram based on HLA-DRB1 allele frequencies (Figure 2) separates all the population in two well-differentiated clusters, one of them shows Amerindians grouped together with North American Na-Dene and Eskimo, as well as Siberians. The other cluster grouped the rest of worldwide populations. Chimila are integrated in the Amerindian group, close to Mexican Mayans. Bootstrap values were 100% in all dendrogram nodes.

Correspondence analysis based on HLA-DRB1 allele frequencies (Figure 3) and on HLA-DRB1 and HLA-DQB1 frequencies conjointly (data not shown) give similar results: Amerindians (including Chimila) form a single and compact group separated from the rest of worldwide populations, which show a continuous genetic variation from Siberia-East Asia to the Mediterranean (Figure 3). The North American Na-Dene and Eskimo seem to be closer to Siberian and Orientals than to Amerindians. Again, genes and languages do not seem to correlate at the microgeographical level (Lewis et al. 2014; Ruhlen 1987), since Amerindian language groups do not correlate with genetic proximity (see Figure 2 and 3) (Arnaiz-Villena et al. 2014b).

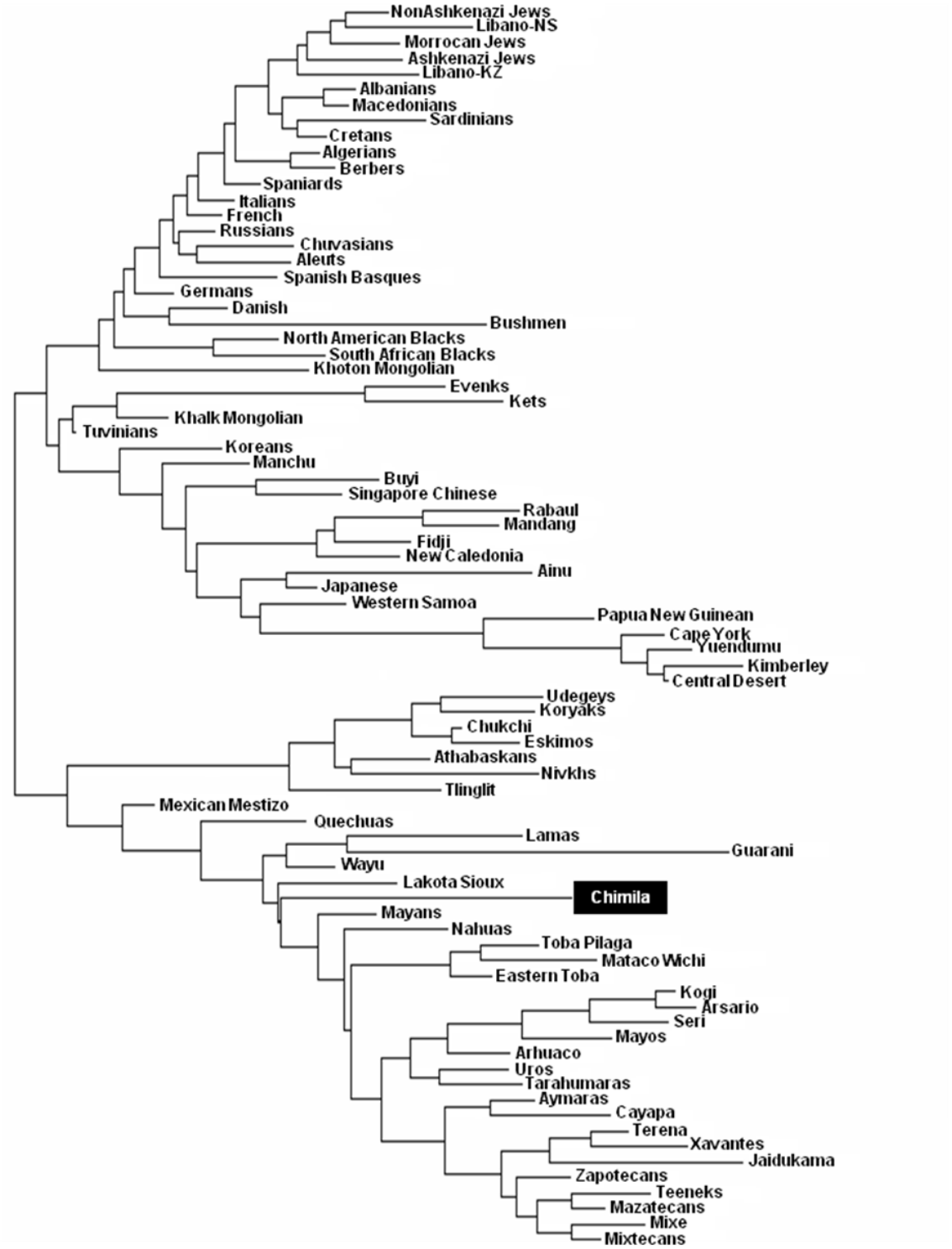

Fig. 2. NJ dendrogram with HLA-DRB1 comparisons of Chimila with other worldwide populations.

Genetic distances between populations (DA) were calculated by using HLA-DRB1 (high resolution). Data from World populations were taken from references stated in Table 1. Amerindians cluster together and separated from the rest of the World populations. Bootstrap values were 100% in all nodes.

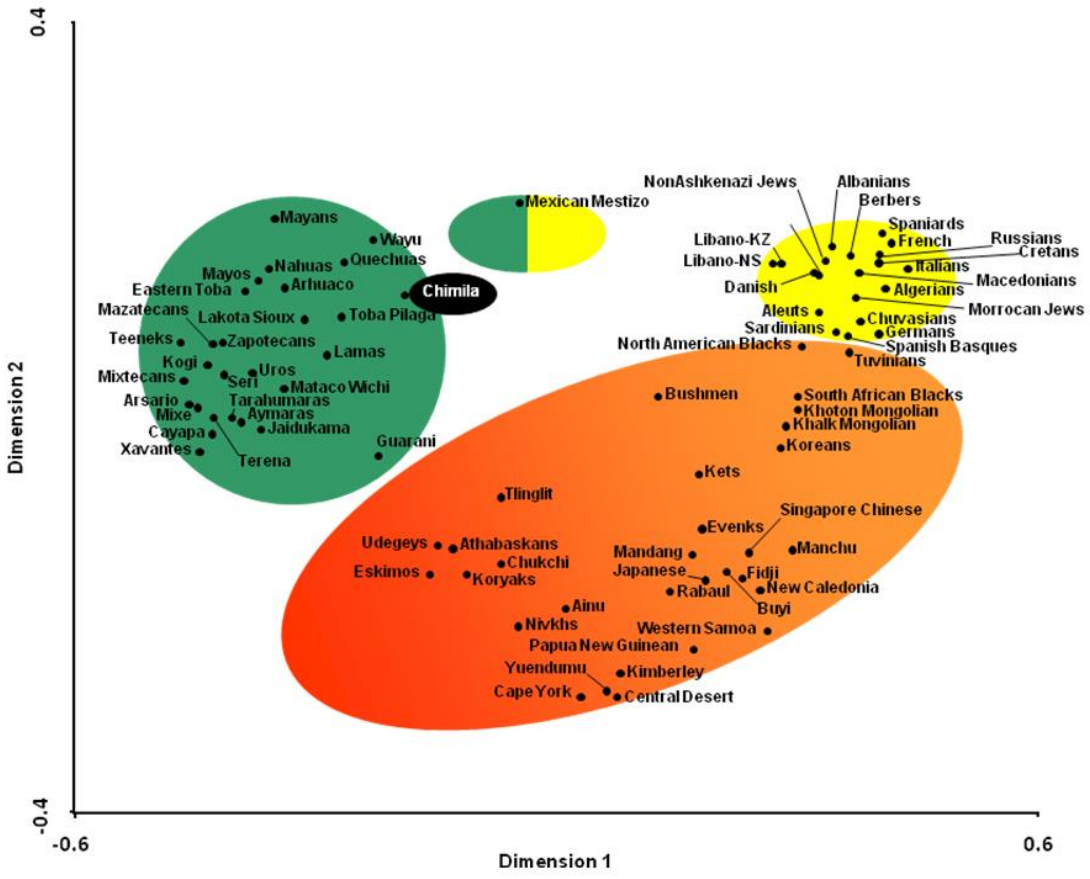

Fig. 3. Correspondence analysis *showing a global view of the relationship between Chimila and Amerindian populations, Oriental and other World populations according to HLA-DRB1 allele frequencies in three dimensions (bidimensional representation)*

HLA-A, -B, -C, -DRB1 and -DQB1 extended haplotypes analysis in Chimila: comparison with other populations

Associations between different HLA loci were estimated. The most probable two loci haplotype combinations (A-B, B-DRB1 and DRB1-DQB1) were calculated (data not shown). The eleven most frequent five HLA loci haplotype combinations (A-B-C-DRB1-DQB1) were calculated and these extended haplotypes are depicted in Table 3; they represent 46.7% of all haplotypes. Chimila extended HLA haplotypes have been obtained for the first time, allowing their comparison with previously reported ones in other

populations (Table 3 and its footnote). The partial class II haplotype DRB1*04:07-DQB1*03:02 is present in association with A*24:02-B*51:10-C*15:02, A*68:01-B*35:43-C*01:02, A*24:02-B*35:43-C*01:02, and A*02:01-B*51:10-C*15:02 (28.7% of total haplotypes). The majority of most frequent extended HLA haplotypes have Amerindian characteristics and they have been found in other Amerindian populations. These five haplotypes are present in high frequency in other Amerindian populations, mainly in Meso-American populations (Mayans, Mayos, Mazatecans, Nahuas, Teeneks) (Arnaiz-Villena et al. 2000; Arnaiz-Villena et al. 2007; Gomez-Casado et al. 2003; Vargas-Alarcon et al. 2006; Vargas-Alarcon et al. 2007) and South-American populations (Aymaras ,Quechuas, Uros) (Arnaiz-Villena et al. 2005; Arnaiz-Villena et al. 2009; Martinez-Laso et al. 2006). The partial class II haplotype typically Amerindian DRB1*16:02-DQB1*03:01 is found in Chimila population in association with A*02:01-B*15:01-C*01:02, also present in Mazatecans (Arnaiz-Villena et al. 2000). A haplotype with no Amerindian characteristics (A*29:02-B*45:01-C*06:02-DRB1*15:01-DQB1*06:02) has been found in this sample. The class II of this haplotype is present in Siberians, Orientals and Caucasoids in high frequencies (Gonzalez-Galarza et al. 2011; Imanishi et al. 1992a). The rest of extended HLA haplotypes (12.7%) have not been found in any other worldwide ethnic group, these haplotypes seem to be specific for this Colombian ethnic group.

In summary, most extended HLA haplotypes in our Chimila sample either are from Amerindian origin or newly found and bearing typical HLA Amerindian alleles.

High frequency Chimila HLA alleles: they are also found in both Pacific and Asian-Pacific populations.

HLA-A*24:02, HLA-C*01:02 and HLA-C*03:04 alleles were found in high frequency in this population (see Table 2); because of this, a World distribution map of these alleles was performed (see Figure 4). Map shows that the highest frequencies of these alleles are found in the Pacific area. Significant differences on these three alleles frequency values were found between Pacific populations and Europe, Africa, continental and West Asia populations, based on t-student test (see Figure 4 footnote).

Table 3. The eleven most frequent HLA-A, -B, -C, -DRB1 and -DQB1 extended haplotypes in the Chimila population.

Haplotype (A-B-C-DRB1-DQB1)	HF (%)	Possible origin
A*24:02-B*51:10-C*15:02-DRB1*04:07-DQB1*03:02[a]	18.1	Amerindian
A*68:01-B*35:43-C*01:02-DRB1*04:07-DQB1*03:02[b]	5.3	Amerindian
A*02:04-B*78:02-C*15:02-DRB1*04:17-DQB1*04:02[c]	4.3	New
A*24:02-B*35:43-C*01:02-DRB1*04:07-DQB1*03:02[d]	3.2	Amerindian
A*29:02-B*45:01-C*06:02-DRB1*15:01-DQB1*06:02[e]	3.2	Asian
A*02:01-B*15:01-C*01:02-DRB1*16:02-DQB1*03:01[f]	2.1	Amerindian
A*02:01-B*51:10-C*15:02-DRB1*04:07-DQB1*03:02[g]	2.1	Amerindian
A*24:02-B*08:50-C*07:01-DRB1*03:01-DQB1*02:01[h]	2.1	New
A*24:02-B*40:09-C*03:04-DRB1*03:02-DQB1*03:01[i]	2.1	New
A*24:02-B*40:09-C*03:04-DRB1*03:02-DQB1*03:02[j]	2.1	New
A*24:03-B*38:01-C*12:03-DRB1*13:01-DQB1*06:03[k]	2.1	New

HF: haplotype frequency

[a]Found without HLA-C in Mayos (3.3%)

[b]Found without HLA-C in Guatemalan Mayans (2.3%), Nahuas (2%), and Uros with HLA-B*35:05 (2.3%)

[c, h, i, j, k]Not found in any other population

[d]Found in USA Hispanic (0.47%), and without HLA-C in Mayans (5.0%), Aymaras (3.1%), Quechua (1.4%), Teeneks (3.7%), Lakota-Sioux with HLA-B*35:01(2.2%), Jaidukama (11.5%) and Mayos (6.0%).

[e]Found in USA Asian (0.04%)

[f] Found without HLA-C in Wayu (5.4%)

[g]Found without HLA-C in Quechuas (1.0%), Jaidukama (1.9%), Lamas with HLA-DRB1*04:11 (1.2%) and Lakota-Sioux with HLA-B*51:01 (1.4%)

References: (Arnaiz-Villena et al. 2005; Arnaiz-Villena et al. 2007; Arnaiz-Villena et al. 2009; Gomez-Casado et al. 2003; Leffell et al. 2004; Martinez-Laso et al. 2006; Martinez-Laso et al. 2011; Moscoso et al. 2006; Petzl-Erler et al. 1997; Silvera et al. 2011; Vargas-Alarcon et al. 2006; Vargas-Alarcon et al. 2007).

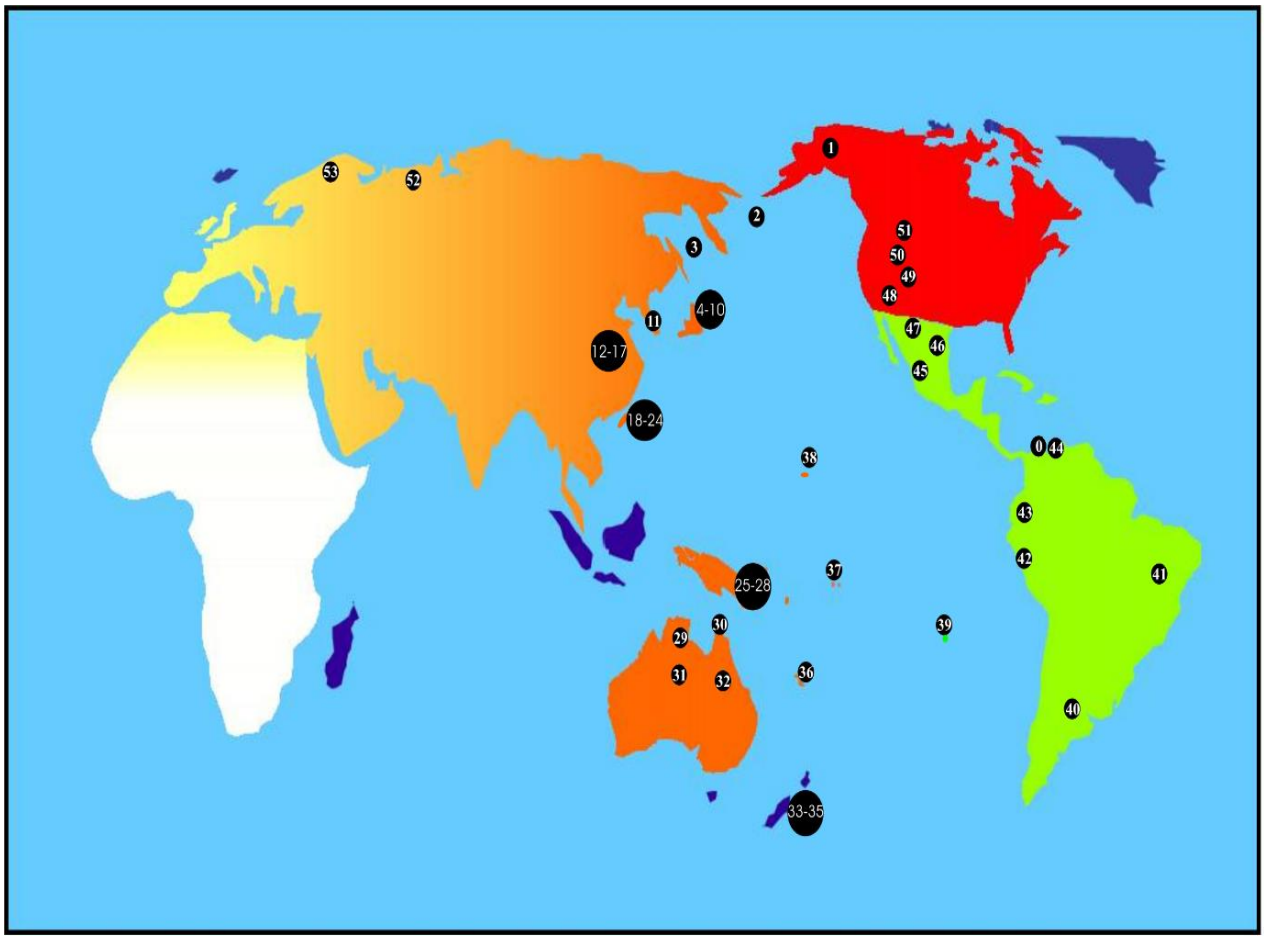

Fig.4. American-Pacific-Asian highest frequency pattern of HLA-A and –C alleles.

HLA alleles less than 10% frequencies have been disregarded. Other HLA-A, -B and –C alleles depicted in Table 2 do not follow an American-Pacific-Asian highest frequency pattern. Populations and common HLA allele highest frequencies:

0- Chimila (Colombia): A*24:02->34%; C*01:02->17%; C*03:04->11.7% (This study)
1- Alaska Yupik (USA): A*24:02->58.1%; C*03:04->38.6% (Leffell et al. 2002).
2- Bering Island Aleut (Russia): A*24:02->24.1%; C*03:04->20% (Moscoso et al. 2008).
3- Sakhalin Island Nivkhi (Russia): C*03:04->32.1% (Lou et al. 1998).
4- Aichi (Japan): C*01:02->20.1% (Gonzalez-Galarza et al. 2015).
5- Kyoto and Osaka (Japan): C*01:02->13% (Kitawaki et al. 2002).
6-Central Japan: A*24:02->37.9; C*03:04->13.7 %(Saito et al. 2000).
7- Japan pop16: A*24:02->36.5%; C*03:04->12.4% (Ikeda et al. 2015).
8- Japan pop3: A*24:02->36.2%; C*03:04->13% (Itoh et al. 2005).
9- Japan pop5: A*24:02->32.7%; C*01:02->17%; C*03:04->11.3% (Tokunaga et al. 1997).
10- Hokkaido Ainu (Japan): A*24:02->24% (Bannai et al. 1996).
11- South Korea: C*03:04->11.6 (Gonzalez-Galarza et al. 2015).
12- Guizhou Province Miao (China): A*24:02->14.7%; C*01:02->24.1%; C*03:04->23.5% (Chen et al. 2007).
13- Guizhou Province Bouyei (China): A*24:02->13.9%; C*01:02->21%; C*03:04->22.3% (Chen et al. 2007).

14- Southwest Dai (China): C*01:02->29.4%; C*03:04->16.5% (Shi et al. 2010)
15- Shanxi (China): C*03:04->15.9% (Gonzalez-Galarza et al. 2015).
16- Guizhou Province Shui (China): A*24:02->24.3%; C*01:02->28.4%; C*03:04->14.7% (Chen et al. 2007).
17- Sichuan (China): A*24:02->17.6%; C*01:02->22.1% (Gonzalez-Galarza et al. 2015)
18- Ami (Taiwan): A*24:02->62.8%; C*01:02->21.9% (Gonzalez-Galarza et al. 2015)
19- Paiwan (Taiwan): A*24:02->86.3%(Gonzalez-Galarza et al. 2015)
20- Tsou (Taiwan): A*24:02->78.4%; C*01:02->11.8% (Gonzalez-Galarza et al. 2015)
21-Rukai (Taiwan): A*24:02->76%(Gonzalez-Galarza et al. 2015)
22- Puyuma (Taiwan): A*24:02->64%(Gonzalez-Galarza et al. 2015)
23- Atayal (Taiwan): A24:02-> 61.8%(Gonzalez-Galarza et al. 2015)
24- Thao (Taiwan): A*24:02->60%; C*01:02->15% (Gonzalez-Galarza et al. 2015)
25- Wosera Abelam (Papua New Guinea): A*24:02->51.3%; C*01:02->10.7%; C*03:04->22.3% (Main et al. 2001).
26- Karimui Plateau Pawaia (Papua New Guinea): A*24:02->74.4%; C*01:02->20%; C*03:04->18.1% (Main et al. 2001).
27- Eastern Highland Goroka Asaro (Papua New Guinea): A*24:02->74.4%; C*01:02->30.2% (Main et al. 2001).
28- Wanigela Keapara (Papua New Guinea): A*24:02->62.7%; C*01:02->13.2% (Main et al. 2001).
29- Kimberly Aborigine (Australia): C*01:02->37.5% (Gao et al. 2000).
30- Cape York Aborigine (Australia): A*24:02->22.3%; C*01:02->18.5% (Gao et al. 2000).
31- Yuendumu Aborigine (Australia): A*24:02->29.8%; C*01:02->24.7% (Gao et al. 2000).
32- Groote Eylandt Aborigine (Australia): A*24:02->29.3%; C*01:02->26.7% (Gao et al. 2000).
33- Polynesians with Full Ancestry (New Zealand): A*24:02->34%; C*01:02->25%; C*03:04->11% (Edinur et al. 2013).
34- Maori with Admixed History (New Zealand): A*24:02->33.3%; C*01:02->31.4%; C*03:04->11.1% (Edinur et al. 2013).
35- Maori Ancestry (New Zealand): A*24:02->38%; C*01:02->45% (Edinur et al. 2013).
36- New Caledonia: A*24:02->60.7%; C*01:02->37.8% (Main et al. 2001).
37- American Samoa: A*24:02-> 33%; C*01:02->24% (Gonzalez-Galarza et al. 2015).
38- Hawaii Okinawa (USA): A*24:02->34.3%; C*01:02->20.5% (Gonzalez-Galarza et al. 2015).
39- Eastern Island (Chile): A*24:02->35.8%; C*01:02->21.4% (Thorsby et al. 2009).
40- Gran Chaco Mataco Wichi (Argentina): A*24:02->10.2% (Cerna et al. 1993).
41- Terena (Brazil): C*03:04->35.1% (Lazaro et al. 1999).
42- Titikaka Lake Uro (Peru): A*24:02->28.1% (Arnaiz-Villena et al. 2009).
43- Cayapa (Ecuador): A*24:02->61.4% (Titus-Trachtenberg et al. 1994).
44- Perja Mountain Yucpa (Venezuela): A*24:02->23.3%; C*01:02->21.2% (Layrisse et al. 2001)
45- Mixtec (Mexico): A*24:02->25.3% (Arnaiz-Villena A et al. 2014b).
46- Oaxaca Mixe (Mexico): A*24:02->24.5%; C*03:04->21.7% (Hollenbach et al. 2001).
47- Chihuahua Tarahumara (Mexico): A*24:02->37.5%; C*01:02->13.6%; C*03:04->39.8% (Garcia-Ortiz et al. 2006).
48- New Mexico Canoncito Navajo (USA): A*24:02->30.5% (Gonzalez-Galarza et al. 2015).
49- Arizona Gila River Amerindian (USA): A*24:02->34.1%; C*03:04->19.5% (Williams et al. 2009).
50- Arizona Pima (USA): A*24:02->36% (Gonzalez-Galarza et al. 2015).
51- South Dakota Lakota Sioux (USA): A*24:02->26.2% (Leffell et al. 2004).
52- Nenet (Russia): C*03:04->14.3% (Evseeva et al. 2002).
53-Northern Sami (Sweden): A*24:02->21.2% (Johansson et al. 2008).
HLA-A*24 was found in low resolution but in high frequency in Jaidukama (Martinez-Laso et al. 2011), in Wayu (Silvera et al. 2011), in Nahua (Vargas-Alarcon et al. 2007), in Mayos (Arnaiz-Villena et al. 2007), in Quechua (Martinez-Laso et al. 2006), in Aymara (Arnaiz-Villena et al. 2005), in Mazatecan (Arnaiz-Villena et al. 2000), Mayan (Gomez-Casado et al. 2003) and Teenek (Vargas-Alarcon et al. 2006) populations.
Other populations from Pacific have frequencies very high of these alleles and their analysis does not add something new (Gonzalez-Galarza et al. 2015).
Statistics comparisons: A*24:02-> Europe-Pacific: $p<0.01$; South Asia-Pacific: $p<0.01$; Western Asia-Pacific: $p<0.01$; North Africa-Pacific: $p<0.01$; Sub-Saharan Africa-Pacific: $p<0.01$. C*01:02-> Europe-Pacific: $p<0.01$; South Asia-Pacific: $p<0.01$; Western Asia-Pacific: $p<0.01$; North Africa-Pacific: $p<0.01$; Sub-Saharan Africa-Pacific: $p<0.01$. C*03:04-> Europe-Pacific: $p<0.05$; South Asia-Pacific: $p<0.01$; Western Asia-Pacific: $p<0.01$; North Africa-Pacific: $p<0.01$; Sub-Saharan Africa-Pacific: $p<0.01$.

The three studied populations bearing HLA-A*24:02 highest frequencies are: Paiwan (Taiwan), Tsou (Taiwan) and Rukai (Taiwan). The three populations having HLA-A*24:02 lowest frequencies are Gran Chaco Mataco Wichi (Argentina), Guizhou Province Bouyei (China) and Guizhou Province Miao (China). The three studied populations bearing HLA-C*01:02 highest frequencies are Maori Ancestry (New Zealand), New Caledonia and Kimberly Aborigine (Australia). The three populations showing HLA-C*01:02 lowest frequencies are Wosera Abelam (Papua New Guinea), Tsou (Taiwan) and Kyoto and Osaka (Japan). The three populations having HLA-C*03:04 highest frequencies are Chihuahua Tarahumara (Mexico), Alaska Yupik (USA) and Terena (Brazil). The three populations showing HLA-C*03:04 lowest frequencies are Polynesians with Full Ancestry (New Zealand), Maori with Admixed History (New Zealand) and Japan pop5. In Spain, Italy, Portugal, France, England and Netherlands populations, these alleles are not found. These particular countries are important to be pointed out because they colonized Pacific areas.

Discussion

Present paper Chimila HLA analysis supports our previous conclusions about Amerindian relationship with other groups and peopling of America, as follows:

1. Amerindians tend to cluster together and separate from other World populations (Figure 2 and 3) (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2000; Arnaiz-Villena et al. 2014a; Gomez-Casado et al. 2003; Rey et al. 2013).
2. Chimila Chibchan language (Ruhlen 1987) does not mean that they are genetically closer to other Chibchan language speaking group (Figures 2, 3, and 4). They are closer to Mayans (Mayan language, Figure 2) and Mayos (Uto-Aztecan speaking group) in some analyses. Also, they stay close to Wayu (Arawak speaking group) (Ruhlen 1987) in correspondence analysis (Figure 3).
3. Chimila HLA frequencies and additional HLA frequencies analyses do not support a three wave's model for America peopling (Arnaiz-Villena et al.

2010; Arnaiz-Villena et al. 2000). However, it is concordant with analyses done by using other genetic markers: mtDNA, Y Chr, or Alu sequences (Horai et al. 1993; Kolman et al. 1996; Merriwether et al. 1996; Torroni et al. 1993).

However, other genetic systems, new genomic methodologies and also a multidisciplinary approach is necessary because only genetics is not enough to accurately approach to America peopling (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014a). It is evident that an ancient founder effect may have disappeared because of a continuous new population admixture effect. Also, Europeans induced that about 85% of America first inhabitants died after 1492 AD because of newly introduced microbes (Dobbins 1993). This may have changed HLA profile of Amerindians after 1492 AD. Stress (like epidemics) causes appearance of new HLA alleles in spermatozoa as shown by single cell PCR (Huang et al. 1995). A set of new alleles may have appeared after 1492 in Amerindians. On the other hand, our present analyses are useful for implementing programs of HLA Pharmacogenomics (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014b; Arnaiz-Villena et al. 2014a), transplantation and preventive medicine of diseases linked to HLA in Chimila and other Amerindians.

This is the first time that HLA-A, -B, -C, -DRB1 and -DQB1 extended haplotypes have been calculated in Chimila. A confirmation that Chimila group has a low admixture degree is that A*24:02-B*51:10-C*15:02-DRB1*04:07-DQB1*03:02 most frequent extended haplotype has a high frequency (18.1%, compared to the second most frequent haplotype A*68:01-B*35:43-C*01:02-DRB1*04:07-DQB1*03:02; which has a frequency of 5.1%). Most haplotypes are of Amerindian origin, since they have already been described (Table 3). However, five new extended haplotypes have been found A*02:04-B*78:02-C*15:02-DRB1*04:17-DQB1*04:02, A*24:02-B*08:50-C*07:01-DRB1*03:01-DQB1*02:01, A*24:02-B*40:09-C*03:04-DRB1*03:02-DQB1*03:01, A*24:02-B*40:09-C*03:04-DRB1*03:02-DQB1*03:02 and A*24:03-B*38:01-C*12:03-DRB1*13:01-DQB1*06:03, which are characteristic of this particular population (Table 3).

On the other hand, A*24:02, C*01:02 and C*03:04 high frequencies were found and also these alleles are present in the most frequent Chimila HLA extended haplotypes. Taking into account our previous detection of HLA alleles and haplotypes shared by Amerindians and Pacific Islanders (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014a), we undertook a comparison of these alleles, which are also frequent in other

Amerindians (Figure 4) (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014a; Gonzalez-Galarza et al. 2015).

These alleles were certainly found with a high frequency (compared to European populations) in some Pacific Ocean populations:

1. A*24:02: Taiwan isolates (86.3% being the maximum frequency), Papua New Guinea (74.4%), New Caledonia (60.7%), and Alaska Yupik (58.1%). Amerindians also presented A*24:02 high frequencies (see Figure 4).
2. C*01:02: New Zealand (45%), New Caledonia (37.8%), Australia Kimberly Aborigine (37.5%), and Papua New Guinea (30.2%) (see Figure 4).
3. C*03:04: Alaska Yupik (38.6%), Sakhalin Island Russia (32.1%), China isolates (23.5% being the maximum frequency) and Papua New Guinea (22.3%) (see Figure 4).
4.

Also, these Pacific A*24:02, C*01:02 and C*03:04 frequencies are significantly higher in Amerindian and Pacific populations when compared to other Europeans, Africans, continental and West Asia populations (see Figure 4 footnote). Altogether (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014a) our results regarding to A*24:02, C*01:02 and C*03:04 alleles study show that HLA support that Pacific Islanders and Amerindians have had genetic exchange (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014a) in ancient times. This exchange may have been bidirectional throughout time between Pacific and American populations. Cultural and other partial genetic traits also show that Amerindians have had genetics contacts with Pacific Easter Island (Arnaiz-Villena et al. 2005; Lie et al. 2007; Thorsby et al. 2009). Our conclusion about Amerindian and Pacific Islands relatedness is that genetic and cultural common traits (Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014a) may have been shared through a both directions traffic; also, European transatlantic genetic and cultural flow is strongly suggested in Paleolithic times (Holden 1999; Stanford and Bradley 2012).

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