Inmunología
Vol. 25 / Núm 1/ Enero-Marzo 2006: 13-24

The uniqueness of amerindians according to HLA genes and the peopling of the Americas

A. Arnaiz-Villena, J. Moscoso, J.I. Serrano-Vela, J. Martinez-Laso

Departamento de Inmunología, Hospital 12 de Octubre, Universidad Complutense, Madrid, Spain.

LA SINGULARIDAD DE LOS AMERINDIOS SEGÚN LOS GENES HLA Y EL POBLAMIENTO DE AMÉRICA

Recibido: 10 de Marzo 2006
Aceptado: 25 de Marzo 2006

RESUMEN

El estudio de la genética de poblaciones se utiliza en epidemiología y en medicina preventiva y predictiva, además de para averiguar el origen y el emparentamiento de los grupos étnicos en estudios antropológicos.

El sistema más polimórfico en humanos es el complejo HLA, y unos pocos de sus alelos están ligados a enfermedad. En este sentido, los marcadores genéticos más utilizados para estudios antropológicos, las variantes de DNA mitocondrial y del cromosoma Y, también están ligadas a enfermedades, pero en un grado comparativamente mayor que HLA, teniendo en cuenta solamente el numero de «alelos sanos» de estos marcadores genéticos. El estudio de los genes HLA demuestra que es muy útil para descubrir el origen y el emparentamiento genético de las poblaciones a nivel mundial. En el presente estudio, hemos comprobado cómo, aparentemente, los Amerindios no se relacionan genéticamente con ninguna otra población del mundo. De acuerdo con los genes HLA, los Amerindios son los primeros habitantes de las Américas y estaban allí cuando llegaron oleadas de gentes hablando lenguas Na Dene (Atabascos, Navajos, Apaches) y Esquimales. Mientras se observa que en todas las otras poblaciones mundiales existe un gradiente de emparentamiento, que va en general concordando con la proximidad o lejanía geográfica, los Amerindios se sitúan aparte de todos. Esta conclusión principal se ha basado en el estudio de 14.968 cromosomas HLA de todo el mundo y en análisis estadísticos utilizando el método de «Neighbour Joining» y los análisis de correspondencia, junto con las distancias genéticas de Nei entre grupos étnicos. El estudio poblacional HLA de Amerindios procedentes de la zona del Océano Pacífico sudamericano es particularmente importante para España, donde se viene registrando un importante flujo migratorio procedente de estos países. Esto hace necesario hacer listas de tipaje HLA con el objeto de efectuar trasplantes entre españoles y Amerindios para fines terapeúticos.

PALABRAS CLAVE: HLA/Amerindios/Diabetes Tipo 1/Transplante/Na Dene/ Esquimales.

ABSTRACT

The genetic profiles of human populations are being used for epidemiology and preventive/predictive medicine, in addition to ascertaining the origin and relatedness of ethnic groups for anthropological studies.

HLA is the most polymorphic genetic system in humans and a few HLA alleles are linked to disease. However, the mtDNA and Chr Y variants, widely used in genetic anthropology, are linked to disease to a much higher degree than HLA, if we only take into account the number of «healthy» alleles from these three different genetic markers.

HLA gene studies allow the determination of the origin and genetic relatedness of worldwide populations. In the present study we have been able to uncover that Amerindians apparently do not relate with any other worldwide population, according to their HLA genetic profile. Amerindians are the very First American Natives that were already in America when Na Dene (Athabascans, Navajo, Apache) and Eskimo speaking people reached it. While other worldwide populations are genetically related following generally a smooth geographic gradient, Amerindians appear apart. This main conclusion has been reached by using 14,968 worldwide HLA chromosomes and Neighbour Joining and correspondence analyses together with Nei´s genetic distances between ethnic groups. The HLA study of southern American Pacific Amerindians is particularly important for Spain, where a recent important immigration of these populations has taken place. This makes it necessary to building up an Amerindian typing list in Spain in order to deal with transplantation between Spaniards and Amerindians for therapeutic uses.

KEY WORDS: HLA/Amerindians/Type 1 diabetes/Transplantation/Na Dane /Eskimo.

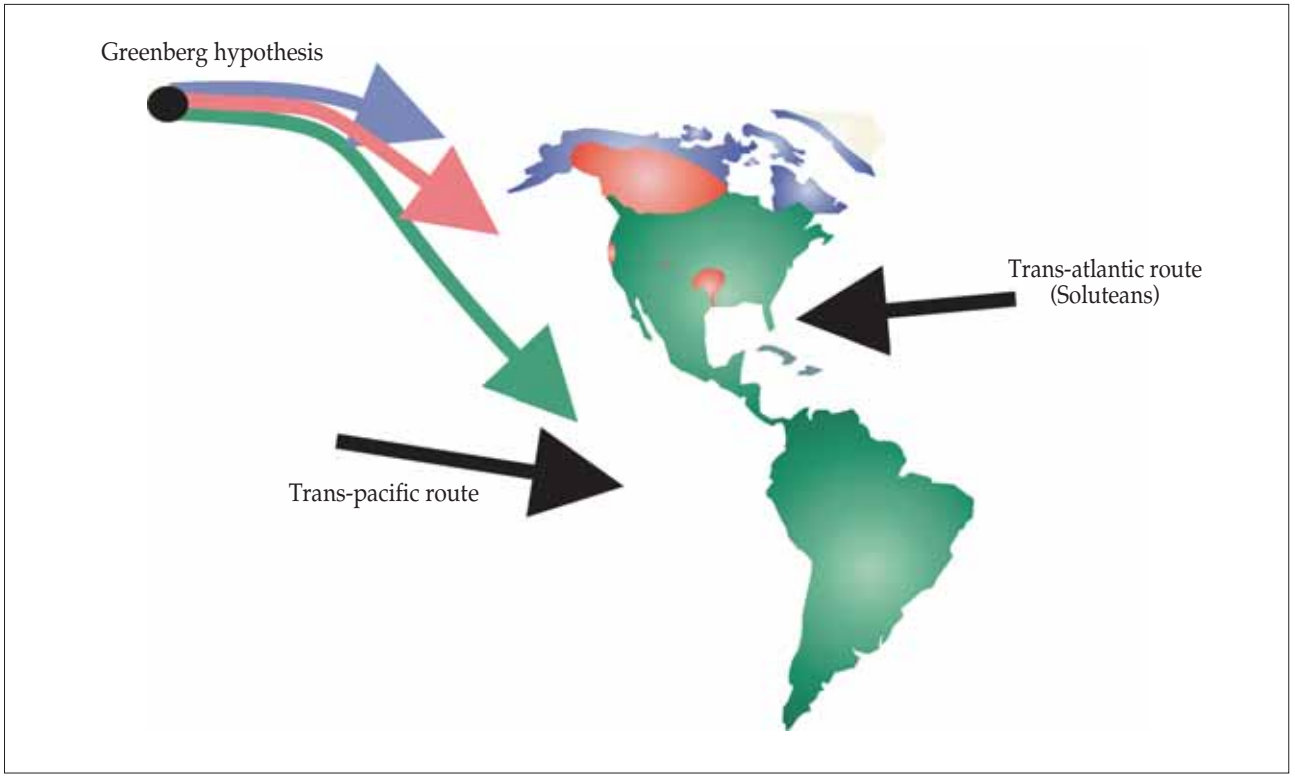

Figure 1. *The peopling of the Americas. Map of America showing different hypothetical migration pathways that could have been followed to reach the American continent. Green: Amerindians. Red: Na Dene peoples. Blue: Eskimos.*

INTRODUCTION

The first Amerindian Natives are believed to have come from Asia through the Bering land bridge between 30,000–12,000 years before the present (BP). These conclusions have been based on cultural, morphological and genetic similarities between American and Asian populations[1]. Both Siberia[1] and Mongolia[2,3] have been put forward as the most likely places of origin in Asia.

Greenberg first postulated the triple migrations theory (Fig. 1) for explaining the peopling of the Americas[4]: Amerindians (most North and South American Indians; 12,000 BP), Na-Dene (Athabascans, Navajo, Apache; 8,000 BP) and Eskimo-Aleuts (6,000 BP). Studies carried out before the widespread use of mitochondrial, Y chromosome and other nuclear DNA markers for the study of populations[5,6] supported the three-wave model, as later did Wallace's mtDNA study[7]. However, more recently other mtDNA studies[8,9] propose that only one wave coming from Mongolia/ North China gave rise to the First Native American ancestors[2,3]. The study of Y chromosome DNA markers seemed to suggest the existence of a single major paternal haplotype in both North and South American Native populations[10,11]. However, more recent studies on the Y chromosome show that more than one paternal founder haplotypes arrived to America during different migrations[12], probably from Siberia[13]. All these discrepancies may be due to methodological (sampling) differences and also to the different genealogical history of each genetic marker and/or to the phylogenetic usefulness of different DNA markers. For instance, functional molecules (mit cyt b) are used against an admixture of intronic and exonic markers, as in the Alu or STR studies: the obtained molecular genetics history could not be the same one.

Alu insertions investigations have also been carried out to ascertain the origin of First Americans[14]. The results are not concordant with the multiple-wave migration hypothesis; instead, three identifiable clusters of people are found, reflecting the geographical distribution. A surprisingly short genetic distance between Chinese and Native Americans was found and explained by a recent gene flow from Asia[14].

A trans-Pacific route of American peopling from Asia or Polynesia has been suggested because HTLV-1 virus strains shared identical sequences in Japan and in the northern coast of South America[15] and some HLA alleles may have been introduced by the same Trans-Pacific route[16].

TABLE I. Populations studied in the present work. A total of 14,698 chromosomes were analyzed. See Fig. 2 for geographical locations

Population	N	Reference	Population	N	Reference
Seri	100	(19)	Manchu	50	(35)
Mixe	55	(19)	Koreans	100	(35)
Mixtecans	103	(19)	Japanese	493	(35)
Zapotecans	75	(19)	Ainu	50	(60)
Lakota Sioux	302	(20)	Khalk Mongolians	100	(61)
Mazatecans	90	(21)	Tuvinians	190	(62)
Teeneks	44	(22)	Khoton Mongolians	85	(61)
Mexican Mestizos	99	Unpublished results	Germans	295	(35)
Mayans	132	(23)	Sardinians	91	(35)
Wayu	88	(24)	Italians	284	(35)
Arhuacs	107	(24)	French	179	(35)
Kogi	42	(24)	Spaniards	176	(63)
Arsario	18	(24)	Spanish Basques	82	(63)
Jaidukama	39	Unpublished results	Algerians	106	(64)
Cayapa	100	(25)	Berbers (Souss)	98	(65)
Lamas	83	(26)	Moroccans	96	(66)
Aymaras	102	(27)	Macedonians	172	(67)
Quechuans	80	(28)	Cretans	135	(68)
Xavantes	74	(16)	Danish	124	(35)
Terena	60	(29)	Russians	200	(69)
Guaranis	32	(19)	Chuvashians	82	(70)
Toba-Pilaga	19	(16)	Fiji Islands	57	(71)
Mataco-Wichi	49	(16)	Papua New Guinean	65	(71)
Eastern Toba	135	(16)	Central Desert	152	(72)
Eskimos	80	(73)	Yuendumu	119	(72)
Athabaskans	124	(74)	Kimberley	82	(75)
Tlingit	53	(35)	Western Samoa	51	(76)
Nivkhs	32	(73)	Madang	65	(71)
Udegeys	25	(73)	Rabaul	60	(71)
Koryaks	92	(73)	New Caledonia	65	(71)
Chukchi	59	(73)	Cape York	80	(75)
Kets	22	(73)	South American Blacks	59	(35)
Evenks	35	(73)	North American Blacks	447	(35)
Singapore Chinese	71	(35)	Hottentots	65	(35)
Buyi	70	(35)	Bushmen	103	(35)

Finally, both genetic[17] and archaeological[18] evidence suggests that a two-ways trans-Atlantic traffic occurred before Columbus discovered America (Fig. 1); archaeologists in New Mexico have recently found tools used 20,000 years ago in Spanish solutrian culture[18].

In the present work, we have studied the North, Meso and South American Amerindians' HLA gene profile and compared it with other North American Indians and worldwide populations. In particular, we have studied: Seri, Mixe, Mixtecans, Zapotecans, Guaranis[19], Lakota Sioux[20] Mazatecans[21], Teeneks[22], Mayans[23], Kogi, Arsario, Arhuacs, Wayu[24], Cayapa[25], Lamas[26], Aymaras[27], Quechuans[28], Terena[29], Xavantes, Toba Pilaga, Mataco Wichi, Eastern Toba[16], Mexican Mestizos and Jaidukama (unpublished results).

Our aims are as follows: 1) To determine the HLA class I (A and B) and class II (DRB1 and DQB1) quasi-specific Amerindian alleles (or more properly allelic lineages; here in after «alleles» for simplicity) by using DNA sequencing and serology; and 2) to compare the Amerindians HLA profile with that of other First American Natives (Na-Dene and Eskimo-Aleuts) and other worldwide populations in order to clarify the much-debated peopling of the Americas and the origins of Amerindians.

Figure 2. *Studied populations. World map showing the location of the populations analysed in the present work and the HLA genetic relationships among themselves according to colour gradient.*

MATERIALS AND METHODS

Population sample

The origin of all populations used for comparisons are detailed in Table I and Fig. 2. 14,698 chromosomes were used for this study, including populations from very different geographical origins: Europeans, Orientals, Polynesians, Micronesians, Na-Dene, Eskimos, Negroids and Amerindians. In particular, the Amerindian group includes tribes from the following linguistic families: Macro-Mixteco (Mixtecan and Zapotecan), Macro-Maya (Mixe), Macro-Yuma (Seris), Chibcha (Arsario, Kogi, Arhuaco and Cayapa), Arawak (Wayu), Ge Pano Caribe (Xavantes, Mataco, Kaingang and Toba) and other Andean groups like Aymara and Quechuas[27,28,30,31]. Amerindian sampling methodology is found in their respective references; non related individuals were used.

HLA typing and DNA sequencing

Samples that have been typed by us in our laboratory, HLA class I (A and B) and HLA class II (DRB1 and DQB1), were performed using a reverse dot-blot technique with the Automated Innolipa system (Innogenetics N.V., Zwijndrecht, Belgium). HLA-A, -B, -DRB1, and -DQB1 allele DNA sequencing was only done in an automated Applied Biosystems ABI-373 DNA sequencer, when this indirect DNA typing yielded ambiguous results[32].

Statistical analysis

Statistical analysis was performed with Arlequin v.2.000 software kindly provided by Schneider et al.[33]. In summary, this program calculated HLA-A, -B, -DRB1, and -DQB1 allele frequencies, Hardy-Weinberg equilibrium and the linkage disequilibrium between two alleles at two different loci. Their level of significance (p) for 2x2 comparisons was determined as previously described[34,35]. In addition, the frequency of maximum likelihood complete presumed haplotypes were deduced from: 1) the 2, 3, and 4 HLA loci haplotype frequencies[34,35]; 2) the previously described haplotypes in other populations[34,35]; and 3) haplotypes if they appeared in two or more individuals and the alternative haplotype was well defined[34,35]. 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 [[36,37], see also Table I]. Non-rooted dendrograms were constructed with the allelic frequencies using the Neighbor-Joining (NJ) method[38] with the genetic distances between

Figure 3. *Neighbor-Joining dendrogram based on HLA-DRB1 allele frequencies. The genetic relatedness among Amerindians, Na-Dene, Eskimos, Asians, Negroids, Europeans and Polynesians are determined by calculating the genetic distances between populations (DA), using HLA-DRB1 allele frequencies. Amerindians cluster together and separated from the rest of the world populations.*

populations [DA,[39]], using DISPAN software comprising the programs GNKDST and TREEVIEW[40,41]. Correspondence analysis in n dimensions and its bidimensional representation was carried out using the VISTA v5.02 computer program [[42], 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 on the display of a statistical visualization of the differences. The heterozygosity analysis was carried out using the «Bottleneck Program»[43] to compare the observed and expected heterozygosity at HLA-A, -B, -DRB1, and -DQB1 loci assuming that all loci fit the Infinity

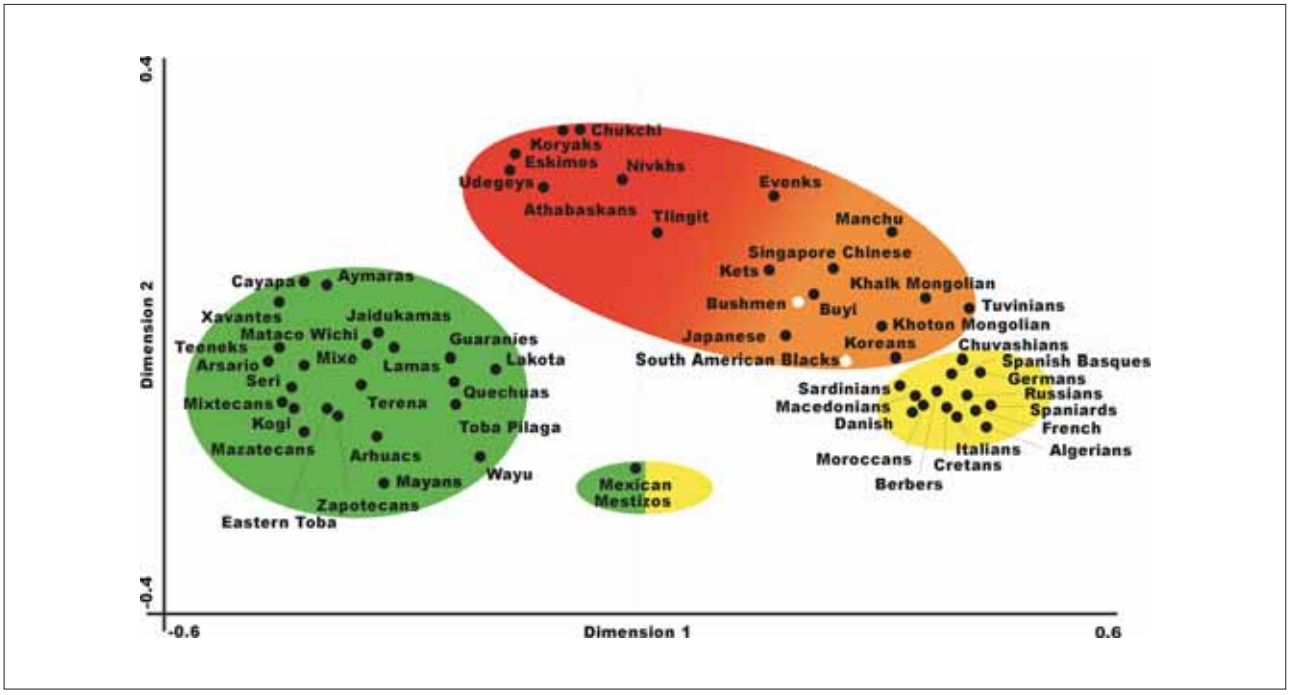

Figure 4. *Correspondence analysis based on HLA-DRB1 and HLA-DQB1 allele frequencies. The analysis shows a global view of the genetic relationships among Amerindian, Na-Dene, Eskimo, Asian, Negroid and European populations according to HLA-DRB1 and HLA-DQB1 allele frequencies. These relationships are calculated in n dimensions and represented in two dimensions. Colours represent an approximate grouping of populations.*

Allele Model (IAM) and mutation-drift equilibrium. Heterozygosity analysis was done as described by Lazaro[29].

RESULTS

Characteristic HLA allele frequencies found in Amerindian populations

The observed allelic frequency values for HLA-A, -B, -DRB1, and -DQB1 loci in Amerindian populations are very homogenous between themselves. We have observed only four HLA-A alleles and four HLA-B alleles with frequencies higher than 5% (A*02, A*24, A*31, A*68, B*35, B*39, B*40 and B*48) in all the Amerindian populations analysed. However, higher resolution DNA typing may yield more alleles.

With regard to the HLA class II alleles, only six alleles for HLA-DRB1 locus had frequencies higher than 5% (DRB1*0404, *0407, *0802, *1402, *1406 and *1602) and are representative for the Amerindian genetic HLA profile. DQB1 allele frequencies reflect the DRB1 locus allele distribution due to the strong linkage disequilibrium between these two loci.

In spite of this, high frequency of HLA-B*48 allele "quasi specific" from Na-Dene (Alaskan and Canadian Athabaskans) and Siberians and HLA-DRB1*0901 found in South Asian populations were also found in a relatively higher frequency than expected in certain Amerindians, like in Quechuans[28], Aymaras[27] and Lamas[26].

Amerindian cluster separately from world populations, according to HLA genes

Two types of analysis were done in order to compare Amerindian populations HLA frequencies with other world population frequencies: 1) with pooled DRB1 and DQB1 data; 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 Amerindian and other populations. Fig. 2 shows that Amerindians (green colour) are separated from other world populations. In turn, all of them are related following a smooth geographic gradient (Fig. 3 and Fig. 4). Fig. 3 depicts an HLA-DRB1 neighbour-joining tree and shows how the Amerindians are grouped together and separated from the Na-Dene and Eskimo Native American groups and also from the Orientals, Negroids, Europeans and Polynesians; this is also seen in the correspondence analysis (results not shown). When we studied the correspondence analyses based on DRB1 and DQB1 allele frequencies between all worldwide populations (except Polynesians,

TABLE II. Most frequent extended haplotypes HLA A-B-DRB1-DQB1 found in Amerindians

Haplotypes	Possible origin
A*02 - B*35 - DRB1*0407 - DQB1*0302[a]	Amerindian
A*02 - B*35 - DRB1*0802 - DQB1*0402[b]	Amerindian
A*68 - B*39 - DRB1*0407 - DQB1*0302[c]	Amerindian
A*24 - B*35 - DRB1*0407 - DQB1*0302[d]	Amerindian
A*24 - B*35 - DRB1*0802 - DQB1*0402[e]	Amerindian
A*02 - B*39 - DRB1*0407 - DQB1*0302[f]	Amerindian
A*31 - B*35 - DRB1*0407 - DQB1*0302[g]	Amerindian
A*24 - B*15 - DRB1*0407 - DQB1*0302[h]	Amerindian
A*68 - B*35 - DRB1*0407 - DQB1*0302[i]	Amerindian
A*02 - B*40 - DRB1*0407 - DQB1*0302[j]	Amerindian
A*02 - B*35 - DRB1*1402 - DQB1*0301[k]	Amerindian
A*24 - B*35 - DRB1*0404 - DQB1*0302[l]	Amerindian
A*02 - B*35 - DRB1*1602 - DQB1*0301[m]	Amerindian
A*68 - B*40 - DRB1*0407 - DQB1*0302[n]	Amerindian

[a]Found in Seri (18.2%); Teeneks (15.5%); Mayans (10.6%); Mixtecans (3%); Mazatecans (2.5%) and Aymaras (1.8%).
[b]Found in Aymaras (10.4%); Mayans (8.4%); Mixtecans (6%); Seri (4.5%); Zapotecans (3%) and Mixe (1.5%).
[c]Found in Mayans (6.4%) and Teeneks (5.2%).
[d]Found in Mayans (5%); Aymaras (3.1%); Seri (2.3%) and Lakota Sioux (2.2%).
[e]Found in Mixtecans (5%); Mayans (4.2%); Teeneks (3.7%); Aymaras (3.1%); Lamas (2.4%); Terena Indians (2.3%) and Seri (2.3%).
[f]Found in Mazatecans (10.8%), Mixe (9%); Mayans (4.2%); Teeneks (3.7%) and Terena Indians (2.3%).
[g]Found in Lakota Sioux (4.1%) and Mayans (2.6).
[h]Found in Seri (2.3%) and Mayans (2.3%).
[i]Found in Quechuans (3.6%) and Mayans (1.5%).
[j]Found in Lamas (5.9%); Aymaras (2.3%) and Mayans (0.7%).
[k]Found in Aymaras (5.5%); Lamas (4.8%); Terena Indians (2.3%); Seri (2.3%) and Mayans (1.1%).
[l]Found in Lakota Sioux (4.3%); Quechuans (2.9%) and Mazatecans (2.5%).
[m]Found in Zapotecans (4.0%); Teeneks (2.9%) and Terena Indians (2.3%).
[n] Found in Terena Indians (4.6%); Teeneks (2.9%) and Lamas (1.8%).
See references (20-23;26-29;35;36;77-80).

Melanesians and Micronesians, due to lack of allele frequencies of DQB1 locus) we could see the same results than the topology of the tree based on DRB1 alone (Fig. 4).

Amerindian specific extended HLA A-B-DRB1-DQB1 haplotypes

The fourteen most frequent extended haploypes found in the Amerindian populations analysed are depicted in Table II. These haplotypes have been demonstrated to be specific of Amerindians; they are present in one or more of those Amerindian isolated groups, but do not appear in any other population of the world.

HLA-DRB1*0407-DQB1*0302 is one of the most frequent class II haplotypes found in many Amerindian populations from Mesoamerica (Seri, Mixe, Mixtecans, Zapotecans, Lakota Sioux, Mazatecans, Teeneks and Mayans) and South America (Aymaras, Quechuans, Lamas and Terena). It is associated with A*02-B*B35, A*68-B*39, A*24-B-35, A*02-B*39, A*31-B*35, A*24-B*15, A*68-B*35, A*02-B*40 and A*68-B40 (see Table II). HLA-DRB1*0802-DQB1*0402 is another typical Amerindian class II haplotype, found in high frequency in several Meso and South American groups (Seri, Mixe, Mixtecans, Zapotecans, Teeneks, Mayans, Lamas, Aymaras and Terena), in association with A*02-B*35 and A*24-B*35 (Table II). Other HLA extended haplotypes frequently found in different Amerindian populations are A*02-B*35-DRB1*1402-DQB1*0301, A*24-B*35-DRB1*0404-DQB1*0302 and A*02-B*35-DRB1*1602-DQB1*0301 (Table II).

Amerindian diabetes haplotypes

HLA-DR4 and HLA-DQB1*0302 are two of the most frequent alleles found in Amerindians, and they are also present in many of HLA extended haplotypes in Amerindian populations. These alleles are associated to type I diabetes in Caucasoid populations[44].

The HLA genes are thought not to confer diabetes susceptibility to Amerindian populations, unless European admixture is present[45,46]. Interestingly, we have studied a case report in an Amerindian Mapuche (Chile) family with a patient affected by diabetes mellitus type I. HLA phenotyping of the members revealed that the haplotypes and both the class I and II genes found were characteristic of Amerindian populations, with no European admixture[47]. However, the HLA diabetogenic factors would be placed outside the HLA class II region since this is shared by all family members, who come from an endogamic Amerindian community. All individuals are HLA-DRB1*0407, HLA-DRB4*0103 and HLA-DQB1*0302. The combination of HLA-A and -B and other closely linked genes present, may be in part responsible for the diabetes development. This is, to our knowledge, the first Amerindian type I diabetes reported case with apparently a recorded non-European (Caucasoid) HLA admixture[47].

DISCUSSION

The peopling of the Americas probably occurred in more than one immigration wave

The relative strength of marker discrimination for explaining the different relatedness found in First Native Americans is difficult to ascertain. However, classical mtDNA and Y markers have given controversial results.

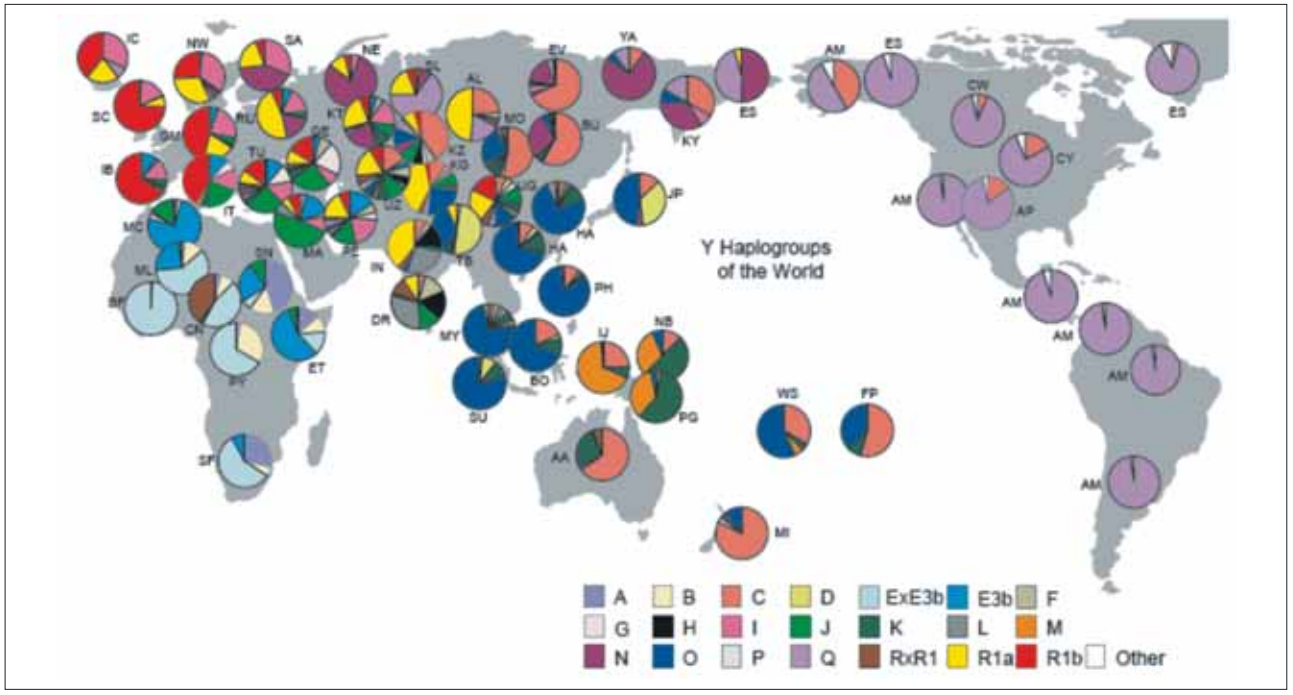

Figure 5. *World distribution of Y chromosome haplogroups. Map showing the location, distribution and relative frequency of Y chromosome haplogroups in different world populations (adapted from http://www.scs.uiuc.edu/~mcdonald/WorldHaplogroupsMaps.pdf). Amerindians show an altogether different haplogroups frequency profile.*

Alu repeats studies have even found a close relatedness between Mesoamericans and Chinese[14]. HTLV-1 virus subtype frequencies in populations suggest close relatedness between Amerindians and Japanese (see Introduction). All of these data should not be disregarded, because all of them should help to account for the true peopling history and First Native Americans' relatedness. In fact, the true scenario may altogether be different and more complicated than foreseen since there are archaeological indications that Caucasoids[48], African Blacks[49], and other populations[17,50] may have all been among the First Native Americans. Particularly, Negroids are reported in the First Spanish Chronicles, as seen by Spanish Conquerors in Meso-America, Caribbean and the north-western area of South America[49]. In fact, many scholars are increasingly doubtful that full-blown Olmec, Toltec, Mayan and Peruvian cultures (otherwise similar to Egyptian culture in certain aspects) appeared without external contact[18,48,49,51]. Moreover, the most ancient archaeological American sites are far from the postulated entrance door: the Bering strait (Monteverde, Chile; Peña Furada, Brazil)[51,52].

Amerindian uniqueness

Our data demonstrate how Amerindians show a relative homogeneity as opposed to other First Native American groups: Figs. 3 and 4 show that Amerindians cluster separately from other North American Indians (Na-Dene and Eskimo-Aleuts). A simple interpretation is that Amerindians are less related to the Na-Dene speaking (Athabaskans) and Eskimo groups than among themselves; it suggests that the Amerindian is a more homogeneous group, which has a different origin from Na-Dene and Eskimo groups. This is also supported by other genetic (classical markers) and cultural data[5]. However, the HLA genetic relatedness among Amerindian groups does not correlate with either geography or language (i.e.: Macro-Mixteco group: Mazatecans, Mixteco, Zapoteco; Macro-Maya: Mixe; Chibcha: Arhuaco, Kogi, Arsario, Cayapa; Andean: Quechua; Andean: Aymara).

Neighbour-Joining and correspondence analyses were done by putting together many worldwide and American populations (Figs. 2, 3, 4). Both analyses show again that Amerindians (Meso and South American) are not genetically related to Na-Dene (Athabaskan) and Eskimos. Also, Amerindians do not show relationships with Polynesians, Australians [almost discarding a massive Pacific colonization, as suggested in[16]], Caucasoids or African Blacks. However, our Bolivian Quechua population shows some HLA relationship with Na-Dene and Asiatic populations (see results), reflecting both possible cultural and genetic contacts with these populations.

On the other hand, Meso and South American Indians could have come from Asia and their HLA antigenic profile could have been changed due to the severe bottleneck that they underwent after the European Invasions in 1492: 80,000,000 people died because of microbia (measles, influenza, smallpox) and war brought by Europeans[53]. It has been proposed that hybrid HLA genes resistant to the European-borne diseases resulted from European-Amerindian contact and subsequent intra-genic gene conversion[54,55]. However, the fact that Amerindians were susceptible to European borne diseases suggests that their original set of HLA molecules was very different to the Eurasian sets. This stresses the true genetic Amerindian uniqueness. In contrast, the STRs high polymorphism found in Amerindians does not support the putative bottleneck for Amerindians, and the low HLA allelic polymorphism may represent a founder effect with little gene flow or an environmentally driven polymorphism[56]. Thus, the problem of the Amerindian origin is still open: they cluster separately from all world populations (Figs. 2, 3, 4). However, Na-Dene North American Indians and Eskimos show an altogether different HLA profile: they are related to some Asian groups (Figs. 3, 4). If Meso and South American Indians come from Asia, they must have originated from a very different Asian people as those existing nowadays, and only a few remains are present in the Quechua population. The physical anthropology of South Amerindians is very different from Asians and also more varied (dolicocephalia, skin colour, etc.)[5]. Indeed, our analysis in Figs. 2, 3 shows that, while representative populations from most world ethnic groups are related, Amerindians cluster into a separate group; correspondence analysis (Fig. 4) also support these findings. The study of chromosome Y haplogroups also supports the uniqueness of Amerindians (Fig. 5). An autochthonous origin for the Amerindians would not fit with the current opinion in science, but multiregional evolution for humans is still hotly supported[57] and mtDNA (which has been the main support for the uni-regional theory of human origins) is now under revision about its ability to calculate divergence times and to support the «out-of-Africa» hypothesis; paternal and maternal mtDNA are both inherited[58].

In addition, the peopling of America sequence may have been more complicated than previously thought: it seems that Caucasoids, Blacks and Mongoloids from China-Mongolia (but not from Siberia, see above) are found to have been in America or the Middle Atlantic (Azores) before Columbus (see Introduction). The possible African and European contacts with Amerindians before Columbus (1492 AD) may not be genetically important; however, the existence of these contacts[17] would help to explain similarities between ancient Egyptian and Mayan-Peruvian civilizations[49].

Finally, Amerindians are also separated from the rest of the world populations on Y chromosome haplogroups bases (Fig. 5). This is consistent with our findings and points out to either an authoctonous origin for the Amerindians (multiregional human evolution theory[59]: South America was an island between about 80-3 million years ago) or to a very long Amerindian isolation. This is not concordant with the theory that present day Amerindians came from Siberia through the Bering Strait. This fact and the recent strong Amerindian immigration into Spain from Pacific South America Amerindians, makes it necessary to build an Amerindian waiting list for transplantation between Spaniards and Amerindians.

ACKNOWLEDGEMENTS

We are grateful for to Prof. M. Serrano-Rios for his help. This work has been financed from Ministerio de Ciencia grant BMC-2001-1299, Ministry of Health grants PI 050338 and PI 051039, and Mutua Madrileña Automovilística grants.

CORRESPONDENCE TO:
Prof. Dr. Antonio Arnaiz-Villena
Departamento de Inmunología
Facultad de Medicina Universidad Complutense
Pabellón 5, planta 4.
Avda. Complutense s/n
28040 Madrid, Spain.
Phone +34 913 941632
E-mail: aarnaiz@med.ucm.es
Web page: http://chopo.pntic.mec.es/biolmol/

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