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HLA Class II Alleles in the Otomi Population of the Mezquital Valley: A Genetic Approach to the History of Interethnic Migrations in the Mexican Central Plateau

Ana Itzel Juárez-Martín,¹ Blanca Zoila González-Sobrino,¹
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ABSTRACT

From a historical and genetic point of view, the Otomi of the Mezquital Valley are a frontier people that have played an important role in the population dynamics of the Mexican Central Plateau. Due to the antiquity of their presence in the area, the Otomi may be bearers of ancient genetic variability, shared mainly today with other groups belonging to the Otomanguean linguistic family and with the Nahuatl. In this study we analyzed the HLA class II allele frequencies reported in Mexican indigenous populations, in order to provide an intraregional-level historical perspective of the genetic relationships between the Otomi of the Mezquital Valley and indigenous populations from other regions of Mexico. We examined genetic variation in *HLA-DRBI* and *-DQBI* loci in 66 nonrelated individuals belonging to seven indigenous communities from the Ixmiquilpan municipality in the Mezquital Valley, in the State of Hidalgo, Mexico. The variability of the *HLA-DRBI* gene among the Otomi of the Mezquital Valley was mainly concentrated in five alleles: *-DRBI*08:02* (31.06%), *-DRBI*04:07* (25.77%), *-DRBI*14:06* (7.55%), *-DRBI*14:02* (6.06%), and *-DRBI*16:02* (4.55%); these alleles have been previously described in other indigenous populations. The most frequent alleles at the *HLA-DQBI* locus were *-DQBI*03:02* (34.09%), *-DQBI*04:02* (31.03%), and *-DQBI*03:01* (19.7%). Furthermore, the *HLA-DQBI*02:02* allele was found in the Otomi group with a frequency of 2.27%; this allele has not been reported in Mexican indigenous populations. In conclusion, the genetic constitution of the Otomi population is intermediate to the northern groups and the genetic variability shared by the peoples of the central regions of Mexico. Furthermore, *HLA-DRBI* and *-DQBI* allelic variability among the Otomi provides insight into the historical processes implied in the biological admixture with European, Asian, and African populations as well as in the admixture with the population of Mexico City associated with long-standing migratory processes.

The Mezquital Valley comprises the western portion of the Mexican State of Hidalgo and is inhabited mainly by Otomi or *Nāhñü* language speakers (Lastra 2006). Information provided by historical, archaeological, and linguistic studies (Garrett 2012) has led to a widespread consensus that Otomi-speaking groups are the oldest inhabitants of the region. Demographic data on the region

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KEY WORDS: HLA CLASS II GENES, OTOMI, MIGRATORY HISTORY, GENETIC VARIABILITY, *DRBI*, *DQBI*.

FIGURE 1. Physiographic map of Mexico: geographic locations of the Mexican populations analyzed in this work. Orographic features are indicated with letters, and studied populations with numbers, as indicated in the key.

KEY

- A** Sierra Madre Occidental mountain range
- B** Northern Arid Plains
- C** Sierra Madre Oriental mountain range
- ◆ Mezquital Valley
- ★ Mexico City

- Mexican Central Plateau
- Climatic Frontier
- Limits State of Hidalgo

1 Seri	8 Mixtec
2 Mayo	9 Zapotec
3 Tarahumara	10 Mazatec
4 Teenek	11 Mixe
5 Otomi	12 Maya (Guatemala)
6 Mazahua	13 Maya (Mexico)
7 Nahua	

(Catálogo de las Lenguas Indígenas Nacionales 2008) reveals that there are approximately 95,057 Otomi speakers in Hidalgo, and 23,261 (~24.5%) reside in the municipality of Ixmiquilpan. Despite intense contact and continuous exchange with regions both north and south of the Mezquital Valley, the Otomi maintain many characteristic traits of Mesoamerican indigenous societies, including the privileged use of the local language, a traditional agrarian lifestyle based on collective ownership of land, and a social organization structured around cohesive domestic groups linked by extensive kinship ties (Báez 2012). Otomi communities in the Mezquital Valley are predominantly but not exclusively endogamous (Oliver et al. 2003, 2005; Franco 1992), and their social and cultural life is organized around a variety of traditional forms of nonmarket exchange (Medina and Quezada 1975), community-based collective labor, and an elaborated rituality, which combines elements of Catholic and pre-Columbian religions (Galinier 1990). Although the Otomi maintain a strong

degree of ethnic identity, they are not an isolated group; on the contrary, they have a long history of migration, first as seminomadic hunter-gatherers and after the Spanish conquest as seasonal laborers in different regions both north and south of the Mezquital Valley (Ruiz de la Barrera 2000).

From a geographical point of view, the Otomi are located on an environmental frontier separating two of the main historical and climatic areas that constitute the Mexican territory: the great Mesoamerican region and the arid plains of northern Mexico, which begin at the Mezquital Valley (at the 21st parallel north) (López Aguilar 2005; Gerhard 1986) (Figure 1). Traditionally, the frontier between these two areas has been regarded as a clear-cut cultural boundary dividing agricultural peoples from hunter-gathering groups (Parsons 1998). Due to the lack of historical sources to reconstruct the history of contacts in this frontier (López Aguilar 1991; Wright 2002), it is often assumed that the two regions developed in relative isolation from each other. Nevertheless, by studying the genetic constitution of the Otomi population, it is possible to reconstruct a more complex and prolonged history of contacts.

The aim of this study is to report the allelic distribution of the HLA class II (*-DRB1* and *-DQB1*) genes among the Otomi population of the Mezquital Valley, as a frontier people who have played a unique role in the shaping of the genetic dynamics of the populations located along the corridor connecting the Northern Plains and the Central Plateau of Mexico. The great diversity observed among these populations is associated with a history of sustained contacts throughout this vast corridor, preserving a permanent dialogue with each other and with other regions during different periods and with different degrees of intensity. Furthermore, formation of the current genetic constitution of Mexican indigenous populations has been dynamic, as it has experienced changes in the distribution of Native American, European, and African alleles throughout time.

The extended collection of genes on the short arm of human chromosome 6 between regions 6p21.31 and 6p21.33 is called the major histocompatibility complex (MHC) and is the most gene-dense region of the human genome. The MHC class II region covers about 0.9 Mb. In the mosaic MHC sequence, 19 class II genes were counted,

including the classical genes *HLA-DP*, *HLA-DQ*, and *HLA-DR*; the nonclassical genes *HLA-DM* and *HLA-DO*; and eight pseudogenes (Vandiedonck and Knight 2009).

One of the outstanding characteristics of the HLA system is its high degree of polymorphism and variability. According to the IMGT/HLA database (www.ebi.ac.uk/imgt/hla/stats.html), a total of 1,582 alleles have been identified for the *HLA-DRB1* locus and 712 for the *HLA-DQB1* locus. At a continental level, American indigenous populations exhibit reduced genetic diversity at the *HLA-DRB1* locus while also exhibiting a number of unique alleles (Watkins et al. 1992; Cerna et al. 1993; Yunis et al. 1994; Trachtenberg et al. 1996; Erlich et al. 1997; Fernández-Viña et al. 1997; Parham et al. 1997; Blagitko et al. 1997; Hollenbach et al. 2001; Gorodezky et al. 2001; Layrisse et al. 2001; Loeza et al. 2002; Arnaiz-Villena et al. 2000, 2007; Zhang et al. 1993). Polymorphisms of the HLA system are relevant in epidemiological terms since certain alleles in populations might have occurred as an adaptive response to the presence of infectious agents or as the effect of genetic drift. Therefore, the extensive allelic polymorphisms and the linkage disequilibrium among different HLA loci are usually used as highly polymorphic genetic markers in anthropological studies (Shen et al. 2010).

We performed an analysis based on genetic distances of *HLA-DRB1* and *-DQB1* genes to provide an intraregional-level perspective of the historical relationship between the Otomi and other Mexican indigenous populations. To study the allelic distribution of *HLA-DRB1* at both regional and worldwide levels, a Bayesian phylogenetic analysis and a principal component analysis (PCA) were also performed.

Materials and Methods

Sample

The sample was composed of 66 nonrelated individuals from seven communities, Peña II, El Tephé, Las Humedades, Cerritos, San Nicolás, Dios Padre, and El Barrido, located in the municipality of Ixmiquilpan in the Mezquital Valley, Mexico (Figure 2). The valley, located at longitude 98°56'42" and 99°51'18" and latitude 19°45'27" and 20°45'9", is part of the Neovolcanic Plateau physiographic

FIGURE 2. Communities of Ixmiquilpan municipality where samples were collected.

- KEY**
- Mezquital Valley
 - Municipality of Ixmiquilpan
 - San Nicolás
 - Cerritos
 - Dios Padre
 - Las Humedades
 - El Tephé
 - Peña II
 - El Barrido

province, situated in the western portion of the Sierra Madre Oriental mountain range (González Quintero 1968).

According to data of the 13th National Population and Housing Census (Instituto Nacional de Estadística y Geografía 2011), the Ixmiquilpan municipality is inhabited by 86,363 people. The average age of the study participants was 49.7 ± 10.6 years. All the volunteers were residents of the Ixmiquilpan municipality with at least two recorded generations of Otomi descent, and most of them spoke *Nāhñü* or Otomi language. After having been informed of the purpose of the study, all the volunteers signed a letter of consent. The present study was approved by the Science and

Bioethics Committee of the National Institute of Respiratory Diseases Ismael Cosío Villegas (approval no. B09-10).

HLA Genotyping

The genomic DNA was obtained using the BDtract Genomic DNA Isolation Kit (Maxim Biotech) from peripheral blood cells collected through venipuncture. High-resolution genotyping of the HLA class II alleles was achieved through PCR with sequence-specific primers (SSPs), which implemented the FastType HLA-DNA SSP Typing System (Bio-synthesis Inc.). This methodology has been accredited and certified by the American Society for Histocompatibility and Immunogenetics.

Statistical Analysis

Allele frequencies were determined by direct counting of molecular data. The statistical analysis was performed using Arlequin software, version 3.0 (Excoffier et al. 2005). The software program was used to test for Hardy-Weinberg equilibrium and to generate genetic distances (F_{ST}). Haplotype frequencies of two-locus combinations were estimated by the maximum likelihood algorithm (Excoffier and Slatkin 1998). The linkage disequilibrium coefficient $\Delta(D)$ (Weir 1979) was calculated according to the standard formula for joint probability for a set of events (Slatkin and Excoffier 1996); standardized $\Delta(D')$ was calculated according to Lewontin (1964).

Comparisons between the fixation indexes F_{ST} and P_{hist} (Φ_{ST}) (Excoffier et al. 1992) were performed with sequences of 1,022 bp obtained from the IMGT/HLA database (release 3.18.0; www.ebi.ac.uk/imgt/hla/). The pairwise allele distances were recorded as the number of nucleotide differences and used for the subsequent calculation of P_{hist} . Since P_{hist} takes into account the nucleotide differences among alleles from a common ancestor, comparisons between F_{ST} and P_{hist} can provide insight into the ancestry and gene flow among populations throughout time. F_{ST} values larger than corresponding P_{hist} values indicate isolation processes more recent than allelic phylogenetic processes and gene flow. P_{hist} values larger than F_{ST} values indicate ancient isolation with less gene flow along history, if any. Phylogeny of *HLA-DRB1* alleles was reconstructed with MrBAYES software, version 3.1.2 (Ronquist and Huelsenbeck 2003) applying the general time-reversible (GTR+G) model of nucleotide substitution. Software was executed for 10 million generations using four Metropolis-coupled Markov chain Monte Carlo chains and the recommended random starting trees. A consensus tree was determined using the 50% majority rule. Principal component analysis (PCA) was performed using the software XLSTAT, version 2014.2.06 (Addinsoft, Inc.), to explore the distribution of *HLA-DRB1* alleles worldwide.

Populations Comparisons

An analysis based on the allele frequencies of HLA class II genes (*-DRB1* and *-DQB1*) was conducted in 12 Mexican indigenous populations and in one sample of admixed population from Mexico City,

as previously reported (Table 1). The populations were organized according to their geographical distribution and grouped as follows: (a) the Sierra Madre Occidental mountain range is occupied by Mayo; Seri; and Tarahumara peoples; (b) the western part of the Sierra Madre Oriental mountain range is inhabited by Teenek or Huastec groups; (c) the Central Plateau consists of the Otomi, Nahua and Mazahua, (d) the Mazatec, Mixe, and Mixtec peoples occupy the mountain areas in Oaxaca; (e) the Zapotec populations are mainly located in the Oaxacan central valleys; and (f) samples of Mayas of Yucatán and Guatemala were added representing southeast region of the country. Although the latter are currently located in a different nation-state, they maintain strong cultural, linguistic, and historical links with the rest of populations settled in the Mexican territory. We included Mexican indigenous and admixed populations, as well as Guatemalan Maya, to perform the intraregional-level analysis. Geographic locations of studied populations are illustrated in Figure 1.

To assess the *HLA-DRB1* allele distribution at a worldwide level, PCA was performed considering a total of 70 populations. Extended references for the populations included in the analysis and the number of individuals in each sample (n) are detailed in Table 1, as follows (numbers in parentheses correspond to the ID in Table 1): the Mexican indigenous populations shown in Figure 1 (1–12), admixed groups from Mexico City (14, 15), and populations from North America (39–42), Central America/Caribbean (13, 16) South America (17–38), Central Asia (57–60), Mediterranean European (61–67), Africa (68–70), and Siberia (43–56).

Results

HLA Class II Alleles in the Otomi Population

Table 2 shows the high-resolution *HLA-DRB1* and *-DQB1* allele frequencies found in the Otomi population of the Mezquital Valley. The diversity of the *HLA-DRB1* gene among the Otomi is distributed in 17 alleles; however, most of the variability is restricted to two alleles: *-DRB1*08:02* (31.06%) and *-DRB1*04:07* (25.77%). The *-DRB1*14:06* allele represents 7.55% of the variation in the Otomi population; *-DRB1*14:02* (6.06%), *-DRB1*16:02* (4.55%), and *-DRB1*04:03*,

Table 1. Populations Included in the Statistical Analysis

ID	Population	n	Location	Reference
1	Otomi	66	Ixmiquilpan, Hidalgo, Mexico	This study
2	Mazahua	65	Estado de México, Mexico	Arnaiz-Villena et al. 2011
3	Nahua	85	Morelos, Mexico	Vargas-Alarcón et al. 2007
4	Mazatec	89	Oaxaca, Mexico	Arnaiz-Villena et al. 2000
5	Mixe	55	Sierra de Oaxaca, Mexico	Hollenbach et al. 2001
6	Zapotec	90	Valle de Oaxaca, Mexico	Hollenbach et al. 2001
7	Mixtec	103	Mixteca Alta, Oaxaca, Mexico	Hollenbach et al. 2001
8	Teenek	55	San Luis Potosí, Mexico	Vargas-Alarcón et al. 2006
9	Mayo	60	Sinaloa, Mexico	Arnaiz-Villena et al. 2007
10	Seri	31	Sonora, Mexico	Alaez et al. 2002
11	Tarahumara	44	Chihuahua, Mexico	García-Ortiz et al. 2006
12	Maya (Mexico)	50	Yucatan, Mexico	Vargas-Alarcón et al. 2011
13	Maya (Guatemala)	132	Quetzaltenango, Guatemala	Gómez-Casado et al. 2003
14	Mexican admixed 1	234	Mexico City, Mexico	Zúñiga et al. 2013
15	Mexican admixed 2	99	Mexico City, Mexico	Vargas-Alarcón et al. 2010
16	Cubans	78	Cuba	Alegre et al. 2007
17	Cayapa	100	Ecuador	Trachtenberg et al. 1994
18	Quechua	80	Bolivia	Martínez-Laso et al. 2006
19	Uros	105	Peru	Arnaiz-Villena et al. 2009
20	Yukpa	73	Venezuela	Layrisse et al. 2001
21	Arsario	18	Colombia	Monsalve et al. 1998
22	Kogi	42	Colombia	Monsalve et al. 1998
23	Arhuaco	107	Colombia	Monsalve et al. 1998
24	Wayuu	88	Colombia	Monsalve et al. 1998
25	East Toba	134	Argentina	Monsalve et al. 1998
26	Mataco Wichi	49	Argentina	Monsalve et al. 1998
27	Toba Pilaga	19	Argentina	Monsalve et al. 1998
28	Chiriguano	56	Argentina	Petzl-Erler et al. 1997
29	Guarani	32	Brazil	Petzl-Erler et al. 1997
30	Kaingang 1	225	Brazil	Petzl-Erler et al. 1997
31	Xavantes	74	Brazil	Monsalve et al. 1998
32	Guarani M'byá	93	Brazil	Tsuneto et al. 2003
33	Guarani Kaiowá	160	Brazil	Tsuneto et al. 2003
34	Guarani Nandeva	87	Brazil	Tsuneto et al. 2003
35	Ache	87	Brazil	Tsuneto et al. 2003

*-DRBI*04:04* are at the same frequency (3.79%). The analysis of the *HLA-DQBI* locus among the Otomi population reveals 10 alleles, although only three of these are present at frequencies higher than 10%: *-DQBI*03:02* (34.09%), *-DQBI*04:02* (31.06%), and *-DQBI*03:01* (19.7%). The Otomi of the Mezquital Valley also exhibit the *HLA-DQBI*02:02* allele at a frequency of 2.27%. It represents the only indigenous population in Mexico that exhibits this allele.

Haplotype Analysis

The two-locus *HLA-DRBI/-DQBI* haplotypes are listed in Table 3. According to the *HLA-DRBI* and *-DQBI* tests of linkage disequilibrium for all pairs of loci, the overall pairwise values (linkage disequilibrium = -260.35 ; linkage equilibrium = -421.060 ; $\chi^2 = 323.25$; 144 df; $P = 0.000$) denote strong linkage disequilibrium. Furthermore, estimates of Hardy-Weinberg equilibrium show a slight deviation from expected/observed heterozygosities (locus

ID	Population	n	Location	Reference
36	Kaingang 2	235	Brazil	Tsuneto et al. 2003
37	Amazonian	41	Brazil	Tsuneto et al. 2003
38	Quechua	44	Brazil	Tsuneto et al. 2003
39	Athabaskan	62	Canada	Monsalve et al. 1998
40	Penutian	26	Canada	Monsalve et al. 1998
41	Tlingit	53	Eskimo-Aleutiano	Monsalve et al. 1998
42	Zuni	50	Eskimo-Aleutiano	Monsalve et al. 1998
43	Evenk	35	Russia (Siberia)	Grahovac et al. 1998
44	Kets	22	Russia (Siberia)	Grahovac et al. 1998
45	Udegey	23	Russia (Siberia)	Grahovac et al. 1998
46	Nivkh	32	Russia (Siberia)	Grahovac et al. 1998
47	Chukchi	59	Russia (Siberia)	Grahovac et al. 1998
48	Eskimos	80	Russia (Siberia)	Grahovac et al. 1998
49	Koryak	92	Russia (Siberia)	Grahovac et al. 1998
50	Mansi	68	Russia (Siberia)	Uinuk-ool et al. 2002
51	Todja	22	Russia (Siberia)	Uinuk-ool et al. 2002
52	Tofalar	43	Russia (Siberia)	Uinuk-ool et al. 2002
53	Buryat	25	Russia (Siberia)	Uinuk-ool et al. 2002
54	Negidal	35	Russia (Siberia)	Uinuk-ool et al. 2002
55	Ulchi	123	Russia (Siberia)	Uinuk-ool et al. 2002
56	Okhotsk Evenki	25	Russia (Siberia)	Uinuk-ool et al. 2002
57	Tuvinians	190	Russia, North Mongolia	Martinez-Laso et al. 2001
58	Khalkha	100	Mongolia	Machulla et al. 2003
59	Oold	52	Mongolia	Machulla et al. 2003
60	Tsaatan	72	Mongolia	Machulla et al. 2003
61	Spaniards (Barcelona)	941	Spain	Vidal et al. 2002
62	Spanish Basques	83	Spain (North)	Sanchez-Velasco et al. 2003
63	Cantabrians	83	Spain (North)	Sanchez-Velasco et al. 2003
64	Cabuernigos	95	Spain (North)	Sanchez-Velasco et al. 2003
65	Pasiegos	88	Spain (North)	Sanchez-Velasco et al. 2003
66	Macedonian	172	Macedonia	Arnaiz-Villena et al. 2001
67	Cretans	135	Crete	Arnaiz-Villena et al. 1999
68	Cameruneses	126	Cameroon	Pimtanonthai et al. 2001
69	Moroccan	98	Morocco	Gómez-Casado et al. 2000
70	Tunisian	104	Tunis	Hajjej et al. 2006

1: expected = 0.8313, observed = 0.8484, P -value = 0.024; locus 2: expected = 0.7469, observed = 0.7723, P -value = 0.136). Linkage disequilibrium and failure to comply with the Hardy-Weinberg law in a population with fewer heterozygotes than expected may be attributed to inbreeding at the community level (Oliver et al. 2003).

The Otomi population exhibits 18 haplotypes, of which the five typical Native American haplotypes are more frequent: *HLA-DRB1*08:02-DQB1*04:02*

(31.06%), *HLA-DRB1*04:07-DQB1*03:02* (25.77%), *HLA-DRB1*14:06-DQB1*03:01* (7.55%), *HLA-DRB1*14:02-DQB1*03:01* (6.06%), and *HLA-DRB1*16:02-DQB1*03:01* (4.55%). The Otomi share eight haplotypes with Mediterranean populations (mainly from Spain, Morocco, Tunis, and Eastern Europe Macedonians): *HLA-DRB1*03:01-DQB1*02:01*, *HLA-DRB1*07:01-DQB1*02:02* and **03:03*, *HLA-DRB1*01:02-DQB1*05:01* (exhibiting the same frequency of 3.03%), *HLA-DRB1*01:03-DQB1*05:01*,

Table 2. Frequencies of the *HLA-DRB1* and *-DQB1* Alleles in the Otomi Population

Allele	Number Observed	Frequency (%)
<i>DRB1</i> *		
01:02	4	3.03
01:03	3	2.27
03:01	4	3.03
04:03	5	3.79
04:04	5	3.79
04:07	34	25.77
04:10	1	0.76
07:01	4	3.03
08:02	41	31.06
12:01	1	0.76
13:01	1	0.76
13:02	1	0.76
14:02	8	6.06
14:06	10	7.55
15:02	3	2.27
15:03	1	0.76
16:02	6	4.55
<i>DQB1</i> *		
02:01	4	3.03
02:02	3	2.27
03:01	26	19.7
03:02	45	34.09
03:03	1	0.76
04:02	41	31.06
05:01	7	5.3
06:01	3	2.27
06:03	1	0.76
06:04	1	0.76

*HLA-DRB1**15:02-*DQB1**06:01 (sharing the frequency of 2.27%), *HLA-DRB1**13:01-*DQB1**03:01, and *HLA-DRB1**13:02-*DQB1**06:04 (both with a frequency of 0.76%), showing clearly the admixture with non-Amerindian populations.

The Otomi also exhibit the same frequency of 3.79% for the haplotypes *HLA-DRB1**04:03-*DQB1**03:02 and *HLA-DRB1**04:04-*DQB1**03:02 that have been reported in several populations worldwide, including Mexican and South American indigenous populations (Yukpa, Arsario, Kogi, Wayuu, Kaigang, Quechuas, and Uros), but also among Asian Mongolians and Mediterranean populations (from Spain, Crete, Morocco, and Tunis) (Vidal et al. 2002; Sanchez-Velasco et al. 2003;

Arnaiz-Villena et al. 1999, 2001; Gómez-Casado et al. 2000; Hajjej et al. 2006; Machulla et al. 2003).

The most probable origin of *HLA-DRB1-DQB1* haplotypes was inferred from data available from previous reports (Zuñiga et al. 2013; Barquera et al. 2008; Yunis et al. 2003; Marsh et al. 1999). Haplotype analysis of *HLA-DRB1-DQB1* genes indicates that approximately 75.75% of the allelic variability among the Otomi is of Native American origin, and 22.73% derives from European alleles shared with other populations. The remaining 1.52% corresponds to haplotypes *HLA-DRB1**12:01-*DQB1**03:01 and *HLA-DRB1**15:03-*DQB1**06:03 of Asian and African origins, respectively.

Population Analysis

Calculations of genetic distances F_{ST} between Otomi and the rest of Mexican populations are shown in Tables 4 and 5. *HLA-DRB1* calculations of F_{ST} in the Otomi reveal that the shortest genetic distances are with the Mazahua (0.017), Mixtec (0.021), and Zapotec (0.029) peoples. There is also a short F_{ST} genetic distance between the aforementioned groups and the rest of the populations of the Mexican Central Plateau. According to the genetic distances F_{ST} estimates for *HLA-DQB1*, after the Mixtec ($F_{ST} = 0.014$) and the Zapotec ($F_{ST} = 0.015$), the closest neighboring groups to the Otomi are the Seri ($F_{ST} = 0.018$) and Tarahumara ($F_{ST} = 0.025$). *HLA-DRB1* and *-DQB1* genetic distances among admixed population from Mexico City and the Otomi ($F_{ST} = 0.019$ and $F_{ST} = 0.015$, respectively) denote the intense gene flow existing between the Otomi and the inhabitants of the central regions of Mexico.

Figure 3 shows comparisons between fixation indices F_{ST} (for recent migrations) and P_{hist} (for ancient migrations) calculated for *HLA-DRB1* and *-DQB1*. The *HLA-DRB1* gene provides evidence that the most intense gene flow occurs between Otomi, Mixtec, Zapotec, and Mazahua groups, all belonging to the same Otomanguan linguistic trunk. The F_{ST} value for Mayo ($F_{ST} = 0.071$) indicates that separation between these and the Otomi was shorter in previous times ($P_{hist} = 0.051$). The Tarahumara is the northern population that has maintained the greatest level of gene flow with the Otomi ($P_{hist} = 0.05$ vs. $F_{ST} = 0.04$). Simultaneously, exchange between Otomi, Nahua, and other groups in the Central Plateau has remained continuous since they settled

Table 3. Two-Locus Haplotype Distribution of *HLA-DRB1-DQB1* in the Otomi Population

HLA-DRB1	HLA-DQB1	No. Cases	Frequency (%)	Δ^a	Δ^{*b}	χ^2
DRB1*01:02	DQB1*05:01	4	3.03	0.0287	1.000	73.6
DRB1*01:03	DQB1*05:01	3	2.27	0.0215	1.000	54.81
DRB1*03:01	DQB1*02:01	4	3.03	0.0294	1.000	132
DRB1*04:03	DQB1*03:02	5	3.79	0.0296	1.000	11.75
DRB1*04:04	DQB1*03:02	5	3.79	0.0296	1.000	11.75
DRB1*04:07	DQB1*03:02	34	25.77	0.1428	0.904	64.54
DRB1*04:10	DQB1*03:02	1	0.76	0.0049	1.000	1.88
DRB1*07:01	DQB1*02:02	3	2.27	0.0220	1.000	98.23
DRB1*07:01	DQB1*03:03	1	0.76	0.0073	1.000	98.23
DRB1*08:02	DQB1*04:02	41	31.06	0.2141	1.000	132
DRB1*12:01	DQB1*03:01	1	0.76	0.0061	1.000	4.31
DRB1*13:01	DQB1*03:01	1	0.76	0.0061	1.000	4.31
DRB1*13:02	DQB1*06:04	1	0.76	0.0075	1.000	132
DRB1*14:02	DQB1*03:01	8	6.06	0.0340	0.691	17.43
DRB1*14:06	DQB1*03:01	10	7.55	0.0538	0.876	35.58
DRB1*15:02	DQB1*06:01	3	2.27	0.0222	1.000	132
DRB1*15:03	DQB1*06:03	1	0.76	0.0075	1.000	132
DRB1*16:02	DQB1*03:01	6	4.55	0.0368	1.000	26.9

All values are significant at $P \leq 0.05$. ^aLinkage disequilibrium coefficient (D). ^bStandardized linkage disequilibrium (D').

Table 4. *HLA-DRB1* F_{ST} Genetic Distances among Mexican Populations of the Study

	Nahua	Mazahua	Teenek	Seri	Tarahumara	Mayo	Mazatec	Mixe	Mixtec	Zapotec	Maya (Guatemala)	Mexican Admix
Mazahua	0.048											
Teenek	0.039	0.086										
Seri	0.098	0.103	0.080									
Tarahumara	0.064	0.065	0.125	0.094								
Mayo	0.081	0.112	0.032	0.055	0.163							
Mazatec	0.018	0.051	0.027	0.088	0.094	0.061						
Mixe	0.035	0.059	0.087	0.120	0.096	0.134	0.034					
Mixtec	0.047	0.045	0.035	0.061	0.082	0.064	0.015	0.019				
Zapotec	0.024	0.041	0.067	0.115	0.071	0.112	0.023	0.018	0.031			
Maya (Guatemala)	0.046	0.061	0.028	0.026	0.093	0.015	0.034	0.081	0.029	0.069		
Mexican Admix	0.016	0.028	0.057	0.098	0.056	0.095	0.024	0.043	0.034	0.018	0.049	
Otomí	0.035	0.017	0.058	0.044	0.041	0.071	0.034	0.042	0.021	0.029	0.035	0.019

All values are significant at $P \leq 0.01$.

in the region, although it has intensified in recent times ($P_{\text{hist}} = 0.04$ vs. $F_{ST} = 0.03$). Finally, according to *HLA-DQB1* there is an ancient genetic divergence between Otomi and Mixe populations ($P_{\text{hist}} = 0.17$). Gene flow between Yucatan Maya and Otomi was more intense in the past ($P_{\text{hist}} = 0.099$) than it is at present times ($F_{ST} = 0.21$).

***HLA-DRB1* Allele Distribution among Mexican Indigenous Populations**

Figure 4 shows a Bayesian phylogeny based on the pairwise allele distances P_{hist} in the *HLA-DRB1* alleles. Forty-seven alleles are present in Mexican indigenous populations. Population-specific alleles account for 32% of the variability, and the

Table 5. *HLA-DQB1* F_{ST} Genetic Distances among Mexican Populations of the Study

	Nahua	Teenek	Seri	Tarahumara	Mayo	Mazatec	Mixe	Mixtec	Zapotec	Maya (Mexico)	Maya (Guatemala)	Mexican Admix
Teenek	0.103											
Seri	0.089	0.082										
Tarahumara	0.049	0.174	0.072									
Mayo	0.101	0.008	0.056	0.16								
Mazatec	0.038	0.027	0.05	0.082	0.032							
Mixe	0.035	0.138	0.084	0.02	0.14	0.05						
Mixtec	0.021	0.079	0.037	0.027	0.072	0.02	0.019					
Zapotec	0.017	0.074	0.038	0.03	0.07	0.016	0.017	0.001*				
Maya (Mexico)	0.045	0.082	0.036	0.055	0.059	0.044	0.075	0.029	0.031			
Maya (Guatemala)	0.058	0.056	0.038	0.086	0.037	0.039	0.098	0.04	0.042	0.167		
Mexican Admix	0.015	0.120	0.065	0.022	0.108	0.049	0.031	0.015	0.016	0.213	0.030	
Otomi	0.042	0.106	0.018	0.025	0.085	0.046	0.043	0.014	0.015	0.021	0.038	0.015

* Not significant ($P > 0.01$). All other values are significant at $P \leq 0.01$.

FIGURE 4. Bayesian phylogeny of *HLA-DRB1* alleles in Mexican indigenous populations. Representative allele frequencies in populations appear on the right side. Populations were geographically ordered following a north-south pattern. Each allele is colored according to its probable origin: red, Native American; blue, Caucasian; green, African; orange, Asian; yellow, Siberian; brown, other populations worldwide; black, uncommon allele.

remaining 68% are shared alleles. Of the groups analyzed, those that exhibit increased allele diversity are the Guatemalan Maya and the Nahua. The *HLA-DRBI* alleles in these two groups represent approximately 70% of the total variability shown in Mexican indigenous populations. Of the populations analyzed, the genetic constitution of the Maya of Guatemala accounts for nearly 60% of the *HLA-DRBI* variation, and the Otomi carry about 40%. The populations carrying the largest number of European alleles are the Maya from Guatemala, the Nahua, and the Otomi of the Mezquital Valley. If we consider the number of nucleotide changes in the sequences, *HLA-DRBI**01:01, *09:02, *10:01, *07:01, *04:02 and *12:01 alleles exhibit the largest number of accumulated differences ($P_{\text{hist}} \sim 0.12$), which suggests the previous divergence of these alleles. All the alleles aforementioned are of European, Asian, or African origin.

Only the Native American alleles -*DRBI**08:02 and *04:07 are shared by all Mexican indigenous populations. Among the Native American alleles, *HLA-DRBI**08:02 has the largest nucleotide diversity, reaching its highest frequency in the northern region of Mexico (35% among the Tarahumara group). Allele -*DRBI**04:07 is found in frequencies greater than 10% among the Mayo, Seri, Teenek, and Maya. The allele -*DRBI**16:02 may be of Mesoamerican origin since it is almost absent in northern populations of Mexico.

Principal Component Analysis

Figure 5 shows a PCA performed for *HLA-DRBI* frequencies, displaying the position of each population in two dimensions. The plot shows that the Otomi belongs to the same cluster as other Mexican indigenous groups and the remaining of American indigenous populations considered in this study. The PCA notably separates the Otomi from admixed populations of Mexico City, who are set between European, African, and Central Asia populations, and the rest of Mexican indigenous populations (including the Otomi), which are closer to the Native American cluster.

Historical Background and Discussion

According to colonial sources, the ancestors of the Otomi were among the earliest agricultural

peoples of the Mexican Central Plateau, as Motolinía (16th century), Torquemada (16th and 17th centuries), Clavijero (18th century), and Orozco y Berra (19th century) state in their studies of the region (Fournier 1996). Although the analysis of *HLA* class II genes does not allow us to assume that the genetic variability of contemporary Mezquital Valley groups corresponds to that of pre-Columbian Otomi populations, it is clear that the valley has been a site of complex genetic interaction as humans have occupied it for the last 11,000 years (Fournier 2012). Data obtained from the analysis of other genetic systems such as mtDNA support the hypothesis of the antiquity of the Otomi of the Mezquital Valley. Sequences of the mtDNA control region (spanning nucleotide positions 16024–16576) suggest that the Otomi were the first people to differentiate from the original genetic pool, which reinforces the claim that the Otomi were among the first settlers in the Mesoamerican territory (Gorostiza et al. 2012). As a result, it is most likely that the Otomi genetic constitution was formed gradually throughout time, by receiving inheritances from different ethnolinguistic groups, as Wright (2005) has suggested based on the study of ancient Mixtec, Mexica (Aztec), and Otomi codex books.

The Otomi territory, which extended from the northern portion of the State of Mexico to the south of the State of Hidalgo, played the role of a buffering zone, dividing the Mexica and Purepecha territories and the lands of those groups generically known as Chichimeca, who occupied the northern portions of Mesoamerica (Parsons 1998). Therefore, the Otomi acted as intermediaries, brokering the relationships between hunter-gathering and agricultural-sedentary peoples. The boundaries shared by Otomi and Chichimeca groups were not fixed. Rather, this was an unstable frontier that was constantly expanding and contracting, but despite its instability, all groups shared a common linguistic background that allowed them to maintain continuous social, economic, cultural, and genetic exchanges (Fournier and Vargas 2002). As the southern continuation of the Chihuahuan deserts, the Mezquital Valley has a strategic geographic location, as it is an unavoidable crossroad connecting northern and central Mexico, which has facilitated contacts among all the cultural regions of the country. At the same time, the Mezquital

Valley serves as a hub, linking the country from the Gulf of Mexico to the Pacific coast (Reyes and Odena 2001). Residing in the frontier, the Otomi share genetic diversity with groups of the Central Plateau and populations beyond the northern border of Mesoamerica. We should bear in mind that the Otomi were capable of moving throughout this vast geographic corridor, a fact that would have facilitated gene flow with groups from the northern arid plains and Mesoamerica. Gene flow between the Otomi and populations from northern Mexico (Tarahumara, Huichol, and Cora) and western Mexico (Purepecha) becomes clear when we analyze the linguistic and historic traits of the Otomi and the data provided by other genetic systems such as mtDNA (Juárez-Martín 2010). The analysis of HLA class II genes reinforces the previous assertion, as it provides evidence of the relative closeness between the Otomi and the Tarahumara and Seri populations from northern Mexico (Tables 4, 5).

The combined use of genetic data with linguistic, geographic, and historical information also reveals that population movements did not take place solely on a north-south axis; migrations and contacts also followed other geographic lines.

The Mazahua and the Otomi share a common linguistic origin but diverged from each other approximately 5.8–7.4 centuries ago (Lastra and Valiñas 2007). The Otomi have the shortest genetic distance with Mazahua ($F_{ST} = 0.018$), demonstrating that gene flow between them has remained relatively stable throughout their history ($P_{hist} = 0.02$; Figure 3). Contacts between Nahua and Otomi populations have been intense and close at least since the 11th century BC, when both groups shared a vast common territory in central Mexico (Obregón 2001). However, after the rise of the Mexica (Aztec) Empire in the 15th century, Nahuatl language spread at the expense of Otomi language, which was gradually displaced and fragmented

FIGURE 5. Principal component analysis showing a global view of the relationship between the Otomi and worldwide populations based on *HLA-DRB1* allele frequencies. Population color code: red, Mexican indigenous (Otomi population is shown with a red triangle); dark blue, Mexican admixed; green, Native American; orange, Central America and Caribe; brown, Eskimo-Aleutian; pink, Asian/Siberian; yellow, Central Asia; light blue, Mediterranean Europeans; purple, African.

into 13 different dialects (Lastra and Valiñas 2007). Nonetheless, this linguistic fragmentation did not interrupt contacts among Otomi groups, and the gene flow was maintained. The genetic distance between Otomi and Nahua ($F_{ST} = 0.035$) can be explained as a result of the intense gene flow that has taken place in the Central Plateau.

The development of lode mining in northern Hidalgo, Zacatecas, and San Luis Potosí after the Spanish Conquest changed the demographics of northern Mexico once more, as it created colonies of Nahua, Spanish, and African slaves, who settled in the Otomi region to work as miners during the 17th century (López Aguilar 2005). These historical processes might explain the presence of some African alleles in the Otomi population as shown in the haplotypes *-DRBI*15:03-DQBI*06:03* and *-DRBI*13:02-DQBI*06:04*. As mentioned before, haplotypes *-DRBI*15:02-DQBI*06:01* and *-DRBI*12:01-DQBI*03:01* can be traced back to the Asian occupation of the Otomi-Mazahua region, probably during colonial or even during recent times (Melville 1994).

HLA-DRBI alleles reported (6 of 17) among the Otomi are of Native American origin, accounting for nearly 75% of the variability, whereas the rest of the alleles reflect a clear admixture with European, Asian, and African populations. It is relevant that all Mexican indigenous populations carry non-Amerindian alleles (Figure 4), although such frequencies are low when contrasted with admixed populations of Mexico (Rubi-Castellanos et al. 2009; Wang et al. 2008; Vargas-Alarcón et al. 2010; Zúñiga et al. 2013). If we compare these values with the admixture calculations reported for *HLA-DRBI* in population of Mexico City, 51.2% of the variability is of Native American origin, 40% has European origin, and the remaining 5% comes from African and Asian regions (Zúñiga et al. 2013).

However, *HLA-DRBI* genetic distances among the Otomi and present-day inhabitants from Mexico City reveal that the genetic exchange is ancient ($P_{hist} = 0.002$), and it has remained intense in present times ($F_{ST} = 0.019$; Figure 3). The closeness between the Otomi and the admixed population of the central valleys is consistent with data reported for other genetic markers such as short-tandem-repeat PCR polymorphisms (Barrot et al. 2005; González-Martín et al. 2008; Hernández Gutiérrez et al. 2005). The Otomi became the

largest indigenous group in Mexico City, reflecting a trend that can be traced back to colonial and pre-Columbian times, but was intensified at the beginning of the 1960s. Toward 1990, over 30% of Mexico City's population comprised immigrants from the states of Hidalgo and Estado de México (Rubio and Zolla 2000: 173).

Although *HLA-DRBI* and *-DQBI* genetic distances between Otomi and mestizo are very short (Tables 4, 5), they do not cluster in the PCA (Figure 5), which shows that the Otomi follow the same genetic pattern of the rest of the Mexican indigenous populations, differing from the admixed populations of Mexico City.

The interethnic dynamics among the populations that settled in the central regions of Mexico can be better understood by taking into account the role of the Otomi as a frontier people that hold a record of gene flow between populations in Northern and Central Mexico. Our intraregional study permits integration of genetic data with linguistic, geographic, and historical information, providing a detailed exploration that enhances the results achieved by analysis conducted at continental or worldwide levels.

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