



Frequency distribution of interleukin-10 haplotypes (-1082 A>G, -819 C>T, and -592 C>A) in a Mexican population

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Genet. Mol. Res. 15 (4): gmr15048411
Received January 11, 2016
Accepted May 24, 2016
Published November 3, 2016
DOI <http://dx.doi.org/10.4238/gmr15048411>

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ABSTRACT. Interleukin 10 (IL-10) is an immunoregulatory cytokine with multiple roles in the immune system. Three single nucleotide polymorphisms at positions -1082 (A>G), -819 (C>T), and -592 (C>A) in the promoter region of the *IL10* gene are believed to be associated with different inflammatory, infectious, and autoimmune diseases. These polymorphisms exhibit a strong linkage disequilibrium (LD) and form three principal haplotypes (GCC, ACC, and ATA). The GCC and ATA haplotypes have been associated with high and low levels of IL-10 production, respectively. The aim of this study was to establish the allele and haplotype frequencies of the *IL10* polymorphisms in Mestizos from western Mexico. SNPs were analyzed in 340 healthy unrelated Mestizos from western Mexico by polymerase chain reaction-restriction fragment length polymorphism. The studied population presented

significant differences, in the distribution of *IL10* polymorphisms, from the Asian, African, and European populations. We also observed a strong LD within -1082 A>G, -819 C>T, and -592 C>A (100% $pc = 7.735 \times 10^{-18}$). The haplotypes ACC (45.4%), ATA (22.0%), GTA (14.9%), and GCC (13.9%) were most frequently observed in this population. The haplotype frequencies, however, differed from those reported previously in Mestizos from central Mexico, Asians, Africans, and European Caucasians, suggesting a differential gene flow in the Mexican Mestizo population. This could account for the genetic variability between Mexicans and populations of other ethnicities. The study of these polymorphisms and their haplotypes could help in expanding our knowledge to design future disease-risk studies on the western Mexican population.

Key words: Mexican Mestizo population; Western Mexico; *IL10* polymorphisms; *IL10* haplotypes; Linkage disequilibrium

INTRODUCTION

Interleukin 10 (IL-10) is an immunoregulatory and pleiotropic cytokine. Human IL-10 is a 37-kDa homodimer, with each monomer comprising 160 amino acids (Asadullah et al., 2003). T regulatory cells, monocytes, macrophages, and T helper cells are the major sources of IL-10; however, IL-10 can also be produced by several other cell types, including natural killer (NK) cells, dendritic cells, cytotoxic T cells, B cells, mast cells, eosinophils, and neutrophils (Saraiva and O'Garra, 2010; Ouyang et al., 2011). The immune regulatory functions of IL-10 are mediated through reduced major histocompatibility complex class II expression, impaired co-stimulation through CD80/CD86, and reduced production of pro-inflammatory cytokines and chemokines, including IL-1 β , IL-6, IL-8, tumor necrosis factor- α , and IL-12 (Pestka et al., 2004; Hedrich and Bream, 2010). However, the biological properties of IL-10 are not limited to inhibition; IL-10 induces B lymphocyte proliferation and differentiation, and promotes immunoglobulin synthesis and antibody production. Likewise, IL-10 activates NK cells and cytotoxic CD8⁺ T lymphocytes (Moore et al., 2001; Mocellin et al., 2003; Hedrich and Bream, 2010).

The *IL10* gene is located at the *IL10* cluster on chromosome 1q32 (Hofmann et al., 2012). Production of interleukin 10 is regulated at both transcriptional and translational levels. Multiple polymorphisms, including three single nucleotide polymorphisms (SNPs) -1082 A>G (rs1800896), -819 C>T (rs1800871), and -592 C>A (rs1800872), that form three major haplotypes (GCC, ACC, and ATA), have been reported in the promoter region of the *IL10* gene (Mege et al., 2006). The GCC and ATA haplotypes, which have been correlated with IL-10 serum levels, have been associated with high and low transcriptional activities, respectively (Suárez et al., 2003; Scarpelli et al., 2006). Considering the role of IL-10 in different pathological processes and the relationship between *IL10* gene polymorphisms and IL-10 production, a number of observational studies have been conducted to investigate the association of *IL10* gene polymorphisms with several diseases. The involvement of IL-10 in inflammatory processes during infection and autoimmunity has been established by several previous studies (Ying et al., 2011; Chen et al., 2013; Qin et al., 2013). Because of its immunosuppressive and anti-inflammatory properties, it has been hypothesized that IL-10 contributes to tumor escape from immune surveillance, thereby enhancing tumor growth (Çil et al., 2014; Qi et al., 2014; Fei et al., 2015; Yu et al., 2015).

Significant genetic differences in people originating from different geographic regions in Mexico could have important implications in the medical community. The geographic structure within the Mexican population influences the interpretation of geographic-pattern susceptibility to various diseases (Rangel-Villalobos et al., 2008). The admixture of Amerindians, Europeans, and Africans over the past 500 years has principally come to shape the present-day gene pool of Mexicans, particularly Mestizos, who represent approximately 93% of the total Mexican population (Rubi-Castellanos et al., 2009). In this study, three polymorphisms in the promoter region of the *IL10* gene were evaluated to determine the prevalent genotypic, allelic, and haplotypic frequencies in western Mexico.

MATERIAL AND METHODS

Subjects

Three hundred and forty unrelated healthy subjects were recruited from the various states of western Mexico, including Nayarit, Jalisco, Colima, and Michoacan. All individuals were Mexican Mestizos or people born in Mexico with a Spanish-derived last name and with Mexican ancestors tracing back three generations, including their own (Rubi-Castellanos et al., 2009). The health status was evaluated according to the medical history of the patients. The following clinical parameters were also determined: complete blood cell count (Cell-Dyn 1700; Abbott Laboratories, Chicago, IL, USA) and complete blood chemical profile (BS120; Mindray, Shenzhen, China). Written informed consent was voluntarily obtained from all participants. The study was performed according to the ethical principles for experiments involving humans dictated in the Declaration of Helsinki, and was approved by the Ethical Investigation and Biosecurity committee of the University Center of Health Sciences of the University of Guadalajara and the Ethics Committee of the Hospital General de Occidente (Registration number: CI/085/2008).

DNA extraction

Genomic DNA (gDNA) was isolated from the peripheral blood leukocytes of healthy subjects collected in EDTA tubes using the modified Miller's technique (Miller et al., 1988).

Genotype analyses

The SNPs -1082 A>G (rs1800896), -819 C>T (rs1800871), and -592 C>A (rs1800872) in the promoter region of the *IL10* gene were genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism. The -1082 A>G polymorphism was amplified using the forward primer 5'-AACACTACTAAGGCTCCTTTGGGA-3', with a mismatch-base (underlined) that could recognize the *Eco*NI restriction enzyme, and the reverse primer 5'-CAAGGAAAAGAAGTCAGGATTCCATGGA-3' (Boiardi et al., 2006). The -819 C>T and -592 C>A polymorphisms were amplified using the primers 5'-CTACTAAGGCTTCTTTGGG-3' (forward) and 5'-AGGTAGTGCTCACCATGACC-3' (reverse) (Santos et al., 2002) and 5'-ATCCAAGACAACACTACTAA-3' (forward) and 5'-TAAATATCCTCAAAGTTCC-3' (reverse), respectively (Mok et al., 1998). PCR was performed in a 25- μ L reaction mixture containing gDNA (1 μ g for -1082 A>G and -819 C>T, and 0.5 μ g for -592C>A), 3 mM each oligonucleotide, 0.5 U/ μ L Taq DNA polymerase, 1X supplied buffer enzyme, 2 mM MgCl₂ (Invitrogen-Life Technologies, Carlsbad, CA, USA),

and 2.5 mM each deoxynucleotide triphosphate (Promega, Madison, WI, USA). The PCR conditions were set as follows: initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s (56°C for *IL10* -592 C>A), extension at 72°C for 30 s, and a final extension at 72°C for 1 min.

One hundred and two- (*IL10* -1082 A>G polymorphism), 348- (*IL10* -819 C>T), and 588-bp (*IL10* -592 C>A) amplified fragments were digested for 3 h at 37°C with 3 U *Eco*NI (*IL10* -1082 A>G: AA, 102 bp; AG, 102, 82, and 20 bp; and GG, 82 and 20 bp), *Msp*I (*IL10* -819 C>T: CC, 288 and 60 bp; CT, 348, 288, and 60 bp; and TT, 348 bp), and *Rsa*I (*IL10* -592 C>A: CC, 305, 233, 42, and 8 bp; CA, 305, 240, 233, 65, 42, and 8 bp; and AA, 240, 233, 65, 42, and 8 bp) (New England Biolabs, Ipswich, MA, USA). The PCR amplified and restriction digested fragments were analyzed by electrophoresis on a 6% polyacrylamide gel for 1 h and subsequently stained with silver nitrate. The digested fragments of the *IL10* -592 C>A polymorphism were analyzed on a 7% polyacrylamide gel for 3 h.

Statistical analysis

The obtained data was statistically analyzed using STATA v.9.2 for Windows (StataCorp., College Station, TX, USA). The allele and genotype frequencies of the *IL10* polymorphisms were obtained by direct counting. The Hardy-Weinberg equilibrium was calculated using the chi-square test, as well as by allele and genotype analysis. Linkage disequilibrium (LD) and the haplotypes of the promoter SNPs -1082 A>G, -819 C>T, and -592 C>A were determined. The haplotype frequencies were inferred using the EMHAPFRE software (Excoffier and Slatkin, 1995). LD was expressed by the Lewontin corrected coefficient (Lewontin, 1964). Comparisons between groups were performed using the Fisher exact test for frequencies <5%. LD in the three loci was assessed according to Gavrillets (Shpak and Gavrillets, 2001). The corrected P (pc) values for multiple tests were adjusted using the Bonferroni correction (Lander and Kruglyak, 1995). Statistical significance was considered at a P value ≤ 0.05 .

RESULTS

Allele and genotype frequencies

The observed and expected frequencies were in Hardy-Weinberg equilibrium ($P > 0.05$). The Mexican Mestizo population from the West showed increased frequencies of the AA genotype (50%) and the A allele (70%) in the *IL10* -1082 A>G polymorphism. The sample population showed a predominant heterozygous genotype (49%) and C allele (~60%) expression in the *IL10* -819 C>T and *IL10* -592 C>A polymorphisms, respectively. The allele and genotype frequencies of the *IL-10* SNPs in Mexican Mestizos from the west were compared to the frequencies reported in a previous study of Mexican Mestizos from the central region (Mexico city) (Vargas-Alarcon et al., 2012), as well as those in populations belonging to the Asian (Liu et al., 2010; Hua et al., 2013), African (Meenagh et al., 2002), and European (Boiardi et al., 2006; Scarpelli et al., 2006) ethnic groups. We observed no differences in the genotypic and allelic frequencies of these SNPs in Mexican Mestizos from the central and western regions. However, a comparison of the Asian population with Mexican Mestizos from the west revealed a marked difference in the allelic and genotypic frequencies of the *IL-10* polymorphisms ($P < 0.001$; Table 1).

The allele and genotype frequencies of the *IL-10* -1082 A>G SNP was found to be similar between the western Mexican and the previously reported European populations.

Similarly, the frequencies of the *IL10* -819 C>T and -592 C>A polymorphisms were similar in Mexican Mestizos from the west and Africans. However, a recessive model of inheritance revealed a greater frequency of the polymorphic allele of the *IL10* -819 C>T polymorphism in the Mexican population (16 vs 6%, P = 0.022, respectively) (Table 1).

Table 1. Polymorphisms in the interleukin promoter region (*IL10* -1082 A>G, -819 C>T, and -592 C>A) of Mexican Mestizos compared to those in other populations.

	Mexican Mestizos		Asian		African	European Caucasian	
	Present study (N = 340) [N (%)]	Vargas-Alarcon et al. (N = 248) [N (%)] ^A	Liu et al. (N = 270) [N (%)]	Hua et al. (N = 102) [N (%)]	Meenagh et al. (N = 86) [N (%)] ^B	Scarpelli et al. (N = 1131) [N (%)]	Boiardi et al. (N = 200) [N (%)] ^C
<i>IL-10</i> -1082 A>G							
Genotype							
AA	169 (50)	125 (50)	240 (89)*	95 (93)*	29 (34) ^κ	485 (43)	83 (42)
AG	139 (41)	106 (43)	27 (10)	7 (7)	49 (57)	516 (46)	90 (45)
GG	32 (9)	17 (7)	3 (1)	0 (0)	8 (9)	130 (11)	27 (13)
Allele							
A	477 (70)	356 (72)	507 (94)*	197 (97)*	107 (62) ^κ	1486 (66) ^κ	256 (64) ^κ
G	203 (30)	140 (28)	33 (6)	7 (3)	65 (38)	776 (34)	144 (36)
Dominant model							
AA	169 (50)	125 (50)	240 (89)	95 (93)	29 (34)	485(43)	83 (42)
AG+GG	171 (50)	123 (50)	30 (11)*	7 (7)*	57 (66) [¶]	646 (57) ^κ	117 (58)
Recessive model							
AA+AG	308 (91)	231 (93)	267 (99)*	102 (100) [¶]	78 (91)	1001 (89)	173 (86)
GG	32 (9)	17 (7)	3 (1)	0 (0)	8 (9)	130 (11)	27 (14)
<i>IL-10</i> -819 C>T							
Genotype							
CC	120 (35)	85 (34)	28 (10)*	6 (6)*	33 (38) ^κ	NR	NR
CT	165 (49)	119 (48)	110 (41)	46 (45)	48 (56)		
TT	55 (16)	44 (18)	132 (49)	50 (49)	5 (6)		
Allele							
C	405 (60)	289 (58)	166 (31)*	58 (28)*	114 (66)	NR	NR
T	275 (40)	207 (42)	374 (69)	146 (72)	58 (34)		
Dominant model							
CC	120 (35)	85 (34)	28 (10)	6 (6)	33 (38)	NR	NR
CT+TT	220 (65)	163 (66)	242 (90)*	96 (94)*	53 (62)		
Recessive model							
CC+CT	285 (84)	204 (82)	138 (51)*	52 (51)*	81 (94) ^κ	NR	NR
TT	55 (16)	44 (18)	132 (49)	50 (49)	5 (6)		
<i>IL-10</i> -592 C>A							
Genotype							
CC	129 (38)	94 (38)	28 (10)*	6 (6)*	33 (38)	615 (54)*	125 (63)*
CA	168 (49)	113 (46)	110 (41)	46 (45)	48 (56)	449 (40)	64 (32)
AA	43 (13)	41 (16)	132 (49)	50 (49)	5 (6)	67 (6)	11 (5)
Allele							
C	426 (63)	301 (61)	166 (31)*	58 (28)*	114 (66)	1679 (74)*	314 (79)*
A	254 (37)	195 (39)	374 (69)	146 (72)	58 (34)	583 (26)	86 (21)
Dominant model							
CC	129 (38)	94 (38)	28 (10)	6 (6)	33 (38)	615 (54)	126 (63)
CA+AA	211 (62)	154 (62)	242 (90)*	96 (94)*	53 (62)	516 (46)*	74 (37)*
Recessive model							
CC+CA	297 (87)	207 (84)	138 (51)*	52 (51)*	81 (94)	1064 (94)*	189 (95) [¶]
AA	43 (13)	41 (16)	132 (49)	50 (49)	5 (6)	67 (6)	11 (5)

Observed and expected frequencies at all polymorphic sites in the study population were in Hardy-Weinberg equilibrium. Allele and genotype frequencies of the *IL10* -1082 A>G, -819 C>T, and -592 C>A polymorphisms in Mestizos from western Mexico were compared to those previously reported in Mexican Mestizo (Vargas-Alarcon et al., 2012), Asian (Liu et al., 2010; Hua et al., 2013), African (Meenagh et al., 2002), and European Caucasian (Boiardi et al., 2006; Scarpelli et al., 2006) populations. ^AThe % of subjects was calculated in the study conducted by Vargas-Alarcon et al. (2012) from the N reported for each genotype and allele. ^BIn the study by Meenagh et al. (2002), the N of subjects was calculated from the reported frequency for each genotype and allele. ^CIn the study by Boiardi et al. (2006), only the -1082 A>G and -592 C>A polymorphisms were genotyped because -819 C>T and -592 C>A polymorphisms were determined to be in complete linkage disequilibrium in the Caucasian population. With reference to studies on other populations analyzed here, the genetic models were inferred using the frequencies reported in each study. Differences in genotype and allele frequencies were compared by the χ^2 test. ^κP < 0.05; [¶]P < 0.01; *P < 0.001; NR: not reported.

Haplotype frequencies and linkage disequilibrium

The linkage disequilibrium between the *IL10* -1082, -819, and -592 sites was individually analyzed for each possible combination; we observed a strong LD (100% $pc = 7.735 \times 10^{-18}$) among the sites. The most frequent haplotypes in Mestizos from western Mexico were: ACC (45.4%), which was used as a reference for haplotype comparisons; ATA (22.0%); GTA (14.9%); and GCC (13.9%). These results are summarized in Table 2.

Table 2. Interleukin 10 (*IL10*) haplotype frequencies in Mexican Mestizos compared to those in other populations.

	Mexican Mestizos		Asian		African	European	
	Present study (N = 340) [N (%)]	Vargas-Alarcon et al. (N = 248) [N (%)]	Liu et al. (N = 270) [N (%)]	Hua et al. (N = 102) [N (%)]	Meenagh et al. (N = 86) [N (%)]	Scarpelli et al. (N = 1131) [N (%)]	Boiardi et al. (N = 200) [N (%)]
Diplotype							
ACC/GTA	79 (23)			NR			
ACC/ATA	77 (23)	NR	NR	41 (40.2)*	NR	NR	NR
ACC/GCC	51 (15)			2 (2)			
ATA/ATA	42 (12)			50 (49)			
ACC/ACC	38 (11)			4 (3.9)			
GCC/GCC	29(9)			0			
Known phase haplotype							
GCC	29 (9)	NR	NR	0 (0)	NR	NR	NR
ACC	38 (11)			4 (4)			
ATA	42 (12)			50 (49)			
Haplotype frequency							
ACC	308 (45.4)	(30.3)**	139 (25.7)*	51 (25)*	(28.0)**	(39.9)*	220 (55.0)*
ATA	149 (22.0)	(38.5)	368 (68.1)	146 (71.6)	(33.9)	(25.7)	36 (9.0)
GTA	102 (14.9)	NR	6 (1.1)	0 (0)	NR	NR	49 (12.3)
GCC	95 (13.9)	(27.2)	27 (5.0)	7 (3.4)	(38.1)	(34.4)	95 (23.7)

Haplotype frequencies of *IL10* polymorphisms in Mestizos from western Mexico were compared to the previously reported frequencies in Mexican Mestizo (Vargas-Alarcon et al., 2012), Asian (Liu et al., 2010; Hua et al., 2013), African (Meenagh et al., 2002), and European (Boiardi et al., 2006; Scarpelli et al., 2006) populations. Differences in haplotype frequencies were compared by the χ^2 test. * $P < 0.001$. **Vargas-Alarcon et al. (2012), Meenagh et al. (2002), and Scarpelli et al. (2006) did not include the number of subjects in their reports (only the haplotype frequencies were included). Known phase haplotypes included all individual carriers of the homozygous genotype for the three loci in the *IL10* gene. NR, not reported.

DISCUSSION

This study was conducted in Mestizos from western Mexico. The National Institute of Anthropology and History defines a Mexican Mestizo as an individual born in Mexico, having lived in the region tracing back three generations, including their own, with a Spanish-derived last name, and a descendant of the original autochthonous inhabitants of the region and of individuals of Spanish, Caucasian, and/or African origin (Rubi-Castellanos et al., 2009). The aim of this study was to establish the gene frequency of polymorphisms and haplotypes in the *IL10* promoter gene in Mestizos from western Mexico. A comparison of the allelic and genotypic frequencies with those reported by a previous study in Mestizos from central Mexico revealed no significant differences between the two population subsets, suggesting a similar genetic distribution of the *IL10* -1082 A>G (rs1800896), -819 C>T (rs1800871), and -592 C>A (rs1800872) polymorphisms in Mestizos from western Mexico and Mestizos from central Mexico (Vargas-Alarcon et al., 2012).

The *IL10* -1082 A>G polymorphism resulted in a 50% homozygous AA and 9%

homozygous GG genotype. On the other hand, Asians showed a comparatively higher AA and lower GG genotype frequency (>89% and 0-1%) than Mestizos from western Mexico (Liu et al., 2010; Hua et al., 2013). Alternatively, the frequency of the heterozygous GA genotype was found to be similar between Mestizos from western Mexico (41%) and African and European populations, as reported in previous studies (Meenagh et al., 2002; Boiardi et al., 2006; Scarpelli et al., 2006). The frequency of the heterozygous genotype CT (49%) at the *IL10* -819 C>T polymorphic site was higher than the frequencies of the homozygous genotypes CC (35%) and TT (16%) in Mexican Mestizos, similar to the frequencies observed in the African and European populations (Meenagh et al., 2002; Boiardi et al., 2006; Scarpelli et al., 2006) and in contrast with the genotype frequencies in Asians [homozygous TT (49%) and CC (~6-10%)] (Liu et al., 2010; Hua et al., 2013). These similarities suggest the presence of a European and African genetic component in the western Mexican regions (Rangel-Villalobos et al., 2008). The genotype frequencies of the polymorphic variants of the *IL10* -592 C>A site in the study population were found to be similar to those previously reported in African and European populations (heterozygous CA, 49%; homozygous CC, 38%; and homozygous AA, 13%) (Meenagh et al., 2002; Boiardi et al., 2006; Scarpelli et al., 2006). On the contrary, a study conducted in Asians showed a higher frequency of the AA genotype (49%) at this polymorphic site (Liu et al., 2010; Hua et al., 2013). These results suggest the lack of a strong Asian influence in the western Mexican region, as previously has been reported (Rangel-Villalobos et al., 2008).

Previous studies have reported that Mestizos from western Mexico have a strong European (60-64%), as well as Amerindian (21-25%) and African (15%) components (Rangel-Villalobos et al., 2008); conversely, the European influence was reported to diminish corresponding to an increase in the Amerindian genotype in the central and southern Mexican regions, while the African influence remained stably low throughout the country (Rubi-Castellanos et al., 2009). The European component of Mexican Mestizos was derived principally from Spanish conquerors, mainly from regions such as Andalucía, León, Extremadura, Castilla Vieja, and Castilla la Nueva. However, a small percent (6%) of the conquerors were also from Portugal and Genova, Italy (Grunberg, 2004). On the other hand, the African Ancestry in Mexican Mestizos was reported to have two origins: from the African slaves introduced by Spanish conquerors, mainly from Cape Verde, Guinea, Angola, and the Congo (Aguirre-Beltrán, 1989) or from Spanish soldiers with Moorish ancestry, resulting from the eight century-long Islamic occupation of the Iberian Peninsula (Gérard et al., 2006; Rubi-Castellanos et al., 2009).

Several studies report a strong LD between the haplotypes of the *IL-10* -1082 A>G, -819 C>T, and -592 C>A sites; the most frequently reported haplotypes included the GCC, ACC, and ATA (Eskdale and Gallagher, 1995). Individual analysis of each of the possible combinations revealed a strong LD between these sites (100% $pc = 7.735 \times 10^{-18}$) in Mestizos from western Mexico, consistent with that reported by previous studies in several populations (Meenagh et al., 2002; Boiardi et al., 2006; Scarpelli et al., 2006; Liu et al., 2010; Vargas-Alarcon et al., 2012; Hua et al., 2013). Haplotype analysis was performed for Mestizos from various states comprising western Mexico, including Nayarit, Jalisco, Colima, and Michoacan; the most frequent haplotypes observed in Mestizos from western Mexico were: ACC (45.4%), ATA (22.0%), GCC (13.9%). Interestingly, we also observed a high frequency of the GTA haplotype (14.9%) in this region of Mexico. High frequencies of the GTA haplotype have been reported in European Caucasian populations (12.3%) (Boiardi et al., 2006), while the same

haplotype is present in low (1.1%) frequencies in the Asian population (Boiardi et al., 2006; Liu et al., 2010). The frequency of this haplotype in Mestizos from western Mexico could support the previous suggestion that there is a European Caucasian influence on the Mexican population.

We have also identified a few differences in the haplotype frequencies between Mestizos from western Mexico (studied population) and those from central Mexico (Vargas-Alarcon et al., 2012). Mestizos from western Mexico showed a higher frequency of the ACC haplotype (45.5%), while those from central Mexico predominantly expressed the ATA haplotype (38.5%). Moreover, the GTA haplotype has not been reported previously in Mexican Mestizos (Fragoso et al., 2011; Vargas-Alarcon et al., 2012). Interestingly, the frequencies reported in central Mexico (Mexico City) are similar to those reported in Asian populations, suggesting a more pronounced Asian influence in this region. Mexico City is the largest urban center in Mexico and comprises a population exceeding 21 million, making it the third largest urban agglomeration in the American continents, and the most populous Spanish-speaking city in the world (G e I Instituto Nacional de Estadística, 2010). Furthermore, central Mexico comprises people from across the country (Lisker et al., 1996). These results indicated the high genetic variability in the Mexican population, thereby hinting at a significant geographic structure within the Mestizo population.

The haplotype analysis revealed a high European influence (ACC haplotype) in Mestizos from western Mexico; on the other hand, we observed a very low African (represented by the GCC haplotype) or Asian (represented by the ATA haplotype) influence on the study population. Scarpelli et al. (2006) and Boiardi et al. (2006) reported a high frequency of the ACC haplotype in Caucasian Italians, similar to that seen in Mestizos from western Mexico. Additionally, Font et al. (2002) also reported a high frequency of the ACC haplotype in Spaniards. In fact, the rampant increase in the interactions with people of European descent, including Italians (Grunberg, 2004) as well as the introduction of African slaves to the continent, generated many Mestizo populations in different parts of the American continent. As a consequence of these events, the indigenous American gene pool has been substantially remodeled over the past 500 years (Schurr and Sherry, 2004). Analysis of classic markers in Mexican-Amerindians allowed for the suggestion that there are no pure Indian groups in Mexico (Lisker et al., 1996). Rangel-Villalobos et al. (2008) explained that the paternal ancestry estimated in western Mexican Mestizos is mainly European (60-64%), followed by Amerindian (21-25%), and African (15%). Significant genetic heterogeneity was established in Mestizos from western (Jalisco State) and northern Mexico (Chihuahua State), compared to that from the central region of the Mexican Republic (Mexico City). This is attributable to a higher percentage of European ancestry in populations from the western and northern regions, than that in populations from the central and southeastern regions, where higher Amerindian ancestry was inferred (Rangel-Villalobos et al., 2008). Analysis of the Y-chromosome and mtDNA in the Mixtec, Zapotec, and Mixes Mexican tribes suggested a differential gene flow, indicating the preferential introduction of European genes by males. The gene flow could be the cause of genetic variability in Mexicans and other populations. Despite this well-established classification, interbreeding between Mexican Mestizos and ethnic groups has resulted in their actual genetic structure to remain largely unknown (Rangel-Villalobos et al., 2008).

A possible limitation of this study is the lack of studies on *IL10* polymorphisms conducted in Mexican tribes from western Mexico, which could potentially supply important information regarding the Amerindian ancestry of *IL10* polymorphisms in western Mexican

Mestizos. Another possible limitation was that the effect of the *IL10* polymorphisms and haplotypes on IL-10 secretion was not assessed in this study; moreover, the selected previous studies analyzing these SNPs in other populations also did not assess this effect (despite the presence of studies reporting this effect).

In conclusion, the *IL10* ACC haplotype frequency was the highest in Mestizos from western Mexico; this was followed by ATA, GCC, and GTA. High frequency of the latter haplotype was also observed in European Caucasians, suggesting a differential gene flow in the Mexican Mestizo population, which could account for the genetic variability between Mexicans and other populations. The study of these polymorphisms and their haplotypes could help in improving our knowledge for future disease-risk studies in the western Mexican population.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by grants provided by CONACYT (Fondo Sectorial SSA/IMSS/ISSSTE-CONACYT, México-Universidad de Guadalajara; Grant #88046 to E. Oregon-Romero and Grant #115567 to C.A. Palafox-Sánchez).

REFERENCES

- Aguirre-Beltrán G (1989). La población negra de México, estudio etnohistórico. In: Fondo de Cultura Económica, México D.F.
- Asadullah K, Sterry W and Volk HD (2003). Interleukin-10 therapy - review of a new approach. *Pharmacol. Rev.* 55: 241-269. <http://dx.doi.org/10.1124/pr.55.2.4>
- Boiardi L, Casali B, Farnetti E, Pipitone N, et al. (2006). Interleukin-10 promoter polymorphisms in giant cell arteritis. *Arthritis Rheum.* 54: 4011-4017. <http://dx.doi.org/10.1002/art.22218>
- Chen XH, Xiong JH, Ning Y, Wen Y, et al. (2013). IL-10 promoter SNPs and susceptibility to leprosy in ethnic groups from southwest China. *Genet. Mol. Res.* 12: 2876-2885. <http://dx.doi.org/10.4238/2013.August.12.3>
- Çil E, Kumral A, Kanmaz-Özer M, Vural P, et al. (2014). Interleukin-10-1082 gene polymorphism is associated with papillary thyroid cancer. *Mol. Biol. Rep.* 41: 3091-3097. <http://dx.doi.org/10.1007/s11033-014-3169-7>
- Eskdale J and Gallagher G (1995). A polymorphic dinucleotide repeat in the human IL-10 promoter. *Immunogenetics* 42: 444-445. <http://dx.doi.org/10.1007/BF00179416>
- Excoffier L and Slatkin M (1995). Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol. Biol. Evol.* 12: 921-927.
- Fei C, Yao XM, Sun Y, Gu XZ, et al. (2015). Interleukin-10 polymorphisms associated with susceptibility to acute myeloid leukemia. *Genet. Mol. Res.* 14: 925-930. <http://dx.doi.org/10.4238/2015.February.2.15>
- Font J, García-Carrasco M, Ramos-Casals M, Aldea AI, et al. (2002). The role of interleukin-10 promoter polymorphisms in the clinical expression of primary Sjögren's syndrome. *Rheumatology (Oxford)* 41: 1025-1030. <http://dx.doi.org/10.1093/rheumatology/41.9.1025>
- Fragoso JM, Vallejo M, Alvarez-León E, Delgadillo H, et al. (2011). Alleles and haplotypes of the interleukin 10 gene polymorphisms are associated with risk of developing acute coronary syndrome in Mexican patients. *Cytokine* 55: 29-33. <http://dx.doi.org/10.1016/j.cyto.2011.03.021>
- Ge I Instituto Nacional de Estadística (2010). Censo General de Población y Vivienda. INEGI, México D.F.
- Gérard N, Berriche S, Aouizérate A, Diéterlen F, et al. (2006). North African Berber and Arab influences in the western Mediterranean revealed by Y-chromosome DNA haplotypes. *Hum. Biol.* 78: 307-316. <http://dx.doi.org/10.1353/hub.2006.0045>

- Grunberg B (2004). El universo de los conquistadores: resultado de una investigación prosopográfica. In: Signos Históricos, Universidad Autónoma Metropolitana-Iztapalapa, México D.F. 12: 94-118.
- Hedrich CM and Bream JH (2010). Cell type-specific regulation of IL-10 expression in inflammation and disease. *Immunol. Res.* 47: 185-206. <http://dx.doi.org/10.1007/s12026-009-8150-5>
- Hofmann SR, Rösen-Wolff A, Tsokos GC and Hedrich CM (2012). Biological properties and regulation of IL-10 related cytokines and their contribution to autoimmune disease and tissue injury. *Clin. Immunol.* 143: 116-127. <http://dx.doi.org/10.1016/j.clim.2012.02.005>
- Hua MC, Chao HC, Yao TC, Lai MW, et al.; PATCH Study Group (2013). Investigation of interleukin-10 promoter polymorphisms and interleukin-10 levels in children with irritable bowel syndrome. *Gut Liver* 7: 430-436. <http://dx.doi.org/10.5009/gnl.2013.7.4.430>
- Lander E and Kruglyak L (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* 11: 241-247. <http://dx.doi.org/10.1038/ng1195-241>
- Lewontin RC (1964). The interaction of selection and linkage. I. general considerations; heterotic models. *Genetics* 49: 49-67.
- Lisker R, Ramírez E and Babinsky V (1996). Genetic structure of autochthonous populations of Meso-America: Mexico. *Hum. Biol.* 68: 395-404.
- Liu J, Song B, Bai X, Liu W, et al. (2010). Association of genetic polymorphisms in the interleukin-10 promoter with risk of prostate cancer in Chinese. *BMC Cancer* 10: 456. <http://dx.doi.org/10.1186/1471-2407-10-456>
- Meenagh A, Williams F, Ross OA, Patterson C, et al. (2002). Frequency of cytokine polymorphisms in populations from western Europe, Africa, Asia, the Middle East and South America. *Hum. Immunol.* 63: 1055-1061. [http://dx.doi.org/10.1016/S0198-8859\(02\)00440-8](http://dx.doi.org/10.1016/S0198-8859(02)00440-8)
- Mege JL, Meghari S, Honstetter A, Capo C, et al. (2006). The two faces of interleukin 10 in human infectious diseases. *Lancet Infect. Dis.* 6: 557-569. [http://dx.doi.org/10.1016/S1473-3099\(06\)70577-1](http://dx.doi.org/10.1016/S1473-3099(06)70577-1)
- Miller SA, Dykes DD and Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215. <http://dx.doi.org/10.1093/nar/16.3.1215>
- Mocellin S, Panelli MC, Wang E, Nagorsen D, et al. (2003). The dual role of IL-10. *Trends Immunol.* 24: 36-43. [http://dx.doi.org/10.1016/S1471-4906\(02\)00009-1](http://dx.doi.org/10.1016/S1471-4906(02)00009-1)
- Mok CC, Lanchbury JS, Chan DW and Lau CS (1998). Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus. *Arthritis Rheum.* 41: 1090-1095. [http://dx.doi.org/10.1002/1529-0131\(199806\)41:6<1090::AID-ART16>3.0.CO;2-6](http://dx.doi.org/10.1002/1529-0131(199806)41:6<1090::AID-ART16>3.0.CO;2-6)
- Moore KW, de Waal Malefyt R, Coffman RL and O'Garra A (2001). Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19: 683-765. <http://dx.doi.org/10.1146/annurev.immunol.19.1.683>
- Ouyang W, Rutz S, Crellin NK, Valdez PA, et al. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu. Rev. Immunol.* 29: 71-109. <http://dx.doi.org/10.1146/annurev-immunol-031210-101312>
- Pestka S, Krause CD, Sarkar D, Walter MR, et al. (2004). Interleukin-10 and related cytokines and receptors. *Annu. Rev. Immunol.* 22: 929-979. <http://dx.doi.org/10.1146/annurev.immunol.22.012703.104622>
- Qi M, Liu DM, Pan LL and Lin YX (2014). Interleukin-10 gene -592C>A polymorphism and susceptibility to gastric cancer. *Genet. Mol. Res.* 13: 8954-8961. <http://dx.doi.org/10.4238/2014.October.31.10>
- Qin B, Wang J, Liang Y, Yang Z, et al. (2013). The association between TNF- α , IL-10 gene polymorphisms and primary Sjögren's syndrome: a meta-analysis and systemic review. *PLoS One* 8: e63401. <http://dx.doi.org/10.1371/journal.pone.0063401>
- Rangel-Villalobos H, Muñoz-Valle JF, González-Martín A, Gorostiza A, et al. (2008). Genetic admixture, relatedness, and structure patterns among Mexican populations revealed by the Y-chromosome. *Am. J. Phys. Anthropol.* 135: 448-461. <http://dx.doi.org/10.1002/ajpa.20765>
- Rubi-Castellanos R, Martínez-Cortés G, Muñoz-Valle JF, González-Martín A, et al. (2009). Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. *Am. J. Phys. Anthropol.* 139: 284-294. <http://dx.doi.org/10.1002/ajpa.20980>
- Santos AR, Suffys PN, Vanderborght PR, Moraes MO, et al. (2002). Role of tumor necrosis factor-alpha and interleukin-10 promoter gene polymorphisms in leprosy. *J. Infect. Dis.* 186: 1687-1691. <http://dx.doi.org/10.1086/345366>
- Saraiva M and O'Garra A (2010). The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10: 170-181. <http://dx.doi.org/10.1038/nri2711>
- Scarpelli D, Cardellini M, Andreozzi F, Laratta E, et al. (2006). Variants of the interleukin-10 promoter gene are associated with obesity and insulin resistance but not type 2 diabetes in Caucasian Italian subjects. *Diabetes* 55: 1529-1533. <http://dx.doi.org/10.2337/db06-0047>

- Schurr TG and Sherry ST (2004). Mitochondrial DNA and Y chromosome diversity and the peopling of the Americas: evolutionary and demographic evidence. *Am. J. Hum. Biol.* 16: 420-439. <http://dx.doi.org/10.1002/ajhb.20041>
- Shpak M and Gavrilets S (2001). Population genetics: Multilocus. eLS, John Wiley and Sons, Chichester.
- Suárez A, Castro P, Alonso R, Mozo L, et al. (2003). Interindividual variations in constitutive interleukin-10 messenger RNA and protein levels and their association with genetic polymorphisms. *Transplantation* 75: 711-717. <http://dx.doi.org/10.1097/01.TP.0000055216.19866.9A>
- Vargas-Alarcon G, Ramirez-Bello J, Juárez-Cedillo T, Ramirez-Fuentes S, et al. (2012). Distribution of the IL-1RN, IL-6, IL-10, INF- γ , and TNF- α Gene Polymorphisms in the Mexican Population. *Genet. Test. Mol. Biomarkers* 16: 1246-1253. <http://dx.doi.org/10.1089/gtmb.2012.0100>
- Ying B, Shi Y, Pan X, Song X, et al. (2011). Association of polymorphisms in the human IL-10 and IL-18 genes with rheumatoid arthritis. *Mol. Biol. Rep.* 38: 379-385. <http://dx.doi.org/10.1007/s11033-010-0119-x>
- Yu YF, Han ZG, Guo WB, Zhang GJ, et al. (2015). Interleukin-10 polymorphisms and nasopharyngeal carcinoma risk: a meta-analysis. *Genet. Mol. Res.* 14: 18945-18957. <http://dx.doi.org/10.4238/2015.December.29.1>