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Analysis of polymorphisms in genes (*AGT*, *MTHFR*, *GPIIIa*, and *GSTP1*) associated with hypertension, thrombophilia and oxidative stress in Mestizo and Amerindian populations of México

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Abstract. Several polymorphisms related to hypertension, thrombophilia, and oxidative stress has been associated with the development of cardiovascular disease. We analyzed the frequency of M235T *angiotensinogen* (*AGT*), A222V *5,10 methylenetetrahydrofolate reductase* (*MTHFR*), *L33P glycoprotein IIIa* (*GPIIIa*), *and I105V glutathione S-transferase P1* (*GSTP1*) *polymor-*

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phisms in 285 individuals belonging to Mexican-Mestizo and five Amerindian population from México, by real time PCR allelic discrimination. Allele and genotype frequencies were compared using χ^2 tests.
All populations followed the Hardy Weinberg equilibrium for assay markers with the exception of the Triki, whose were in Hardy Weinberg dysequilibrium for the *glutathione S-transferase P1* polymorphism.
Interestingly, according to all the analyzed single nucleotide polymorphisms (SNPs), the Triki population was the most differentiated and homogeneous group of the six populations analyzed. A comparison of our data with those previously published for some Caucasian, Asian and Black populations showed quite significant differences. These differences were remarkable with all the Mexican populations having a lower frequency of the 105V allele of the *glutathione S-transferase P1* and reduced occurrence of the 222A allele of the *5,10 methylenetetrahydrofolate reductase*. Our results show the genetic diversity among different Mexican populations and with other racial groups.

Keywords: Gene polymorphisms, *AGT*, *GPIIIa*, *GSTP1*, *MTHFR*, Mexican populations

1. Introduction

Several epidemiological and clinical studies have reported associations between polymorphisms of various genes related to hypertension, thrombophilia, and oxidative stress with development of cardiovascular and cerebrovascular diseases. Among these genes are *angiotensinogen* (*AGT*) (MIM 106150) [1], *glycoprotein IIIa* (*GPIIIa*) (MIM 173470) [2], *5,10 methylenetetrahydrofolate reductase* (*MTHFR*) (MIM 607093) [3], and *glutathione S-transferase P1* (*GSTP1*) (MIM 134660) [4]. However, other studies have shown no evidence of association between these polymorphisms and risk of these diseases [1,5,6].

Approximately 30% to 40% of the population variability in blood pressure is genetically determined [7]. The importance of the renin-angiotensin system for maintenance of normal cardiovascular homeostasis is well established [8]. It has been suggested that individuals with a p.M235T (c.704C>T) polymorphism in the *AGT* gene in the homozygous TT state have increased plasma angiotensinogen levels and a corresponding increase in risk of hypertension [9]. Likewise, it was demonstrated that moderate hyperhomocysteinemia is considered as an independent risk factor for ischemic cardiovascular disease [10]. The genetic basis of this disease may be due to a polymorphism in the *MTHFR* gene (p.A222V, c.677C>T) where homozygosity for the C-677T substitution results in reduced MTHFR enzyme activity and subsequently elevated homocysteine concentrations of ∼20% [11].

GPIIIa is a thrombophilic gene involved in the regulation of vascular thrombosis. The GPIIb/GPIIIa complex mediates platelet aggregation by acting as a receptor for fibrinogen. This complex also acts as a receptor for von Willebrand factor and fibronectin [12]. The polymorphism c.98C>T in this gene causes a p.L33P substitution and the existence of two antigenically distinct forms of the mature GPIIb/IIIa antigen on platelets [13]. This variant itself has been associated with risk of premature acute coronary syndromes and stroke in young Caucasian women [14]. In addition, oxidative stress is thought to play an important role in the pathophysiology of hypertension, although this statement lies within the context of the development of preeclampsia [15]. It has been hypothesized that reduced levels of GSTP1 due to the the lower activity of the *GSTP1* (p.I105V, c.313A>G) allele in this syndrome may be an indicator of decreased capacity of the GST detoxification system and may cause a prolonged exposure to reactive by-products, which may contribute to maternal endothelial dysfunction [15,16].

It has been observed that diverse genetic polymorphisms associated with cardiovascular diseases are usually found in most human populations but often with variations in the allele frequencies [17]. The Mexican-Mestizo population is constituted by a mixture of Europeans and Africans with native Indian subjects [18]. These individuals have a proportion of 56% Amerindian genes, 40% Caucasian genes, and 4% African genes [19]. According to the National Institute of Anthropology, a Mexican-Mestizo is defined as a person who was born in Mexico, has a Spanish-derived last name, and has a family of Mexican ancestors back to the third generation [20]. Moreover, in Mexico ∼7% of the total population corresponds to an ethnic group, using the language as a classification criterion [21]. Interestingly, several of these groups have maintained a limited admixture level with the Mestizo population as a consequence of the geographical isolation and/or cultural barriers.

Because the Mestizos and Amerindian populations are genetically heterogeneous and considering the putative role of polymorphisms in genetic susceptibility to cardiovascular diseases, the principal aim of this study was to analyze the frequency of p.M235T *an-*

Fig. 1. Geographical location of the six Mexican populations analyzed in the study. 1. Yaqui, 2. Huichol, 3. Puépecha, 4. Triki, 5. Maya, 6. Mestizo.

giotensinogen, p.A222V *5,10 methylenetetrahydrofolate reductase*, p.L33P *glycoprotein IIIa*, and p.I105V *glutathione S-transferase P1 polymorphisms* in the Mestizo and five Amerindian populations from México.

2. Subjects

The study was approved by the Institute's Human Research Committee. Informed consent was obtained from all subjects before participating in the study. The local authorities of the Amerindian population gave their approval to participate in the study, and a translator was used as needed. From the 285 subjects, DNA from blood samples was obtained from apparently normal male individuals with no phenotypic abnormalities. Individuals ranged in age from 18–60 years ages. The study was comprised of subjects from six Mexican populations from six different geographical regions (Fig. 1): 71 Mexican-Mestizos living in Mexico City or its surroundings; 29 Purépechas from the state of Michoacán in western Mexico; 27 Yaquis from the state of Sonora located in northern Mexico; 15 Huicholes from Nayarit in northwestern Mexico; 89 Trikis from the state of Oaxaca and 54 Mayas from the state of Yucatán, both located in southeastern México.

The Mexican-Mestizo group resulted from the admixture between Native American and European (Spanish) populations with a much smaller contribution of African groups. Because México City has been a site of massive immigration during the last century receiving inhabitants from all around the country, this group can be considered representative of the overall Mexican population. Only individuals born in México whose parents and grandparents were born in México were considered Mexican-Mestizo. All Amerindian individuals and their ancestors throughout three generations were born in the same community and spoke their own native language.

3. Methods

3.1. Genotyping

The study was performed to determine the frequencies of the polymorphisms in *AGT* (p.M235T), *MTHFR* (p.A222V),*GPIIIa* (p.L33P), and *GSTP1* (p.I105V) genes. Peripheral blood samples were obtained from all individuals, and genomic DNA was purified by standard techniques [22]. Single nucleotide polymorphism (SNP) analysis was performed using real-time PCR allelic discrimination TaqMan assays (AB) with minor modifications. All PCR reactions contained 20 ng of DNA, 2.5 μl TaqMan Universal Master Mix (AB) (2X), 0.25 μl primers and probes (10X) and water for a final volume of 5 μl, including the appropriate negative controls in all assays. Real-time PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of amplification (95 °C for 15 s and 62 °C for 1 min). For each cycle, the software determined the fluorescent signal from the VIC or FAM-labeled probe

Table 1
Primers and probes used for the PCR allelic discrimination TaqMan assay

Gene	Primers	Probe
AGT	5'-GCTGTGACAGGATGGAAGACT-3′ (sense)	VIC 5′-CTGGCTCCC**A**TCAGG-3′ wt
	5′-AGTGGACGTAGGTGTTGAAAGC-3′ (antisense)	FAM 5′-CTGGCTCCC**G**TCAGG-3′ mt
MTHFR	5′-GCACTTGAAGGAGAAGGTGTCT-3′ (sense)	VIC 5′-CTGGCTCCC**A**TCAGG -3′ wt
	5′-CCTCAAAGAAAAGCTGCGTGATG-3′ (antisense)	FAM 5′-CTGGCTCCC**G**TCAGG-3′ mt
GPIIIa	5′-TCTCTTTGGGCTCCTGTCTTACA-3′ (sense)	FAM 5′-TGAGCCC**A**GAGGCA-3′ wt
	5′-CAGATTCTCCTTCCGGTCACA-3′ (antisense)	VIC 5′-TGAGCCC**G**GAGGCA-3′ mt
GSTP1	5′-CCTGGTGGACATGGTGAATGAC-3′ (sense)	VIC 5′-CTGCAAATAC**A**TCTCC-3′ wt
	5′-CAGATGCTCACATAGTTGGTGTAGA-3′ (antisense)	FAM 5′-CTGCAAATAC**G**TCTCC-3′ mt

Table 2
Allele frequencies of the *MTHFR* c.677C>T, *AGT* c.704C>T, *GPIIIa* c.98C>T and *GSTP1* c.313A>G polymorphisms in several populations

		Huichol	Maya	Purépecha	Trikis	Yaquis	Mestizo	Caucasian	Asian	Black
SNP	Allele					Frequency				
MTHFR	C	0.467[a]	0.389[b]	0.293[a]	0.078[a]	0.537[a]	0.472[a]	0.68[26]	0.55[27]	0.9[28]
C→T	T	0.533	0.611	0.707	0.922	0.463	0.528	0.32	0.45	0.1
								(2n = 820)	(2n = 936)	(2n = 658)
AGT	**C**	0.733[c]	0.88[c]	0.879[c]	0.983[d]	0.759[c]	0.775[c]	0.41[40]	0.81[38]	0.83[39]
C→T	T	0.267	0.12	0.121	0.017	0.241	0.225	0.59	0.19	0.17
								(2n = 200)	(2n = 1028)	(2n = 806)
GPIIIa	C	1.0[c]	0.973[e]	0.949[c,f]	1.0[e]	0.907[f]	0.958[e,f]	0.83[14]	0.99[41]	0.89[42]
C→T	T	0.0	0.027	0.051	0.0	0.093	0.042	0.17	0.01	0.11
								(2n = 432)	(2n = 632)	2n = 92)
GSTP1	A	0.143[g]	0.38[g]	0.413[g]	0.298[g]	0.352[g]	0.415[g]	0.64[43]	0.83[44]	0.58[45]
A→G	G	0.857	0.62	0.587	0.702	0.648	0.585	0.36	0.17	0.42
								(2n = 236)	(2n = 626)	(2n = 274)

Note:The distributions of allele frequencies obtained in our populations (Amerindian and Mexican Mestizos) were compared to those previously described in other populations (Caucasian, Asian and Black population) using the chi-square test (p significative values were in the range of $p <$ 0.05 to $p < 0.0001$).
[a]Decreased frequency when compared to Caucasian and Black populations
[b]Decreased frequency when compared to Caucasian Asian and Black populations.
[c]Increased frequency when compared to Caucasian population
[d]Increased frequency when compared to Caucasian, Asian, and Black populations.
[e]Increased frequency when compared to Caucasian, and Black populations.
[f]Decreased frequency when compared to Asian population.
[g]Decreased frequency when compared to Caucasian, Asian, and Black population.

(Applied Biosystems). Allelic discrimination was performed using specific primers and probes for each allele. The sequences of the primers and probes for each polymorphism are shown in Table 1.

3.2. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS v.10 (SPSS, Chicago, IL, USA). Continuous variables were compared by unpaired Student's test. Genotype distributions were tested for deviation from Hardy-Weinberg equilibrium in all groups. Allele and genotype frequencies were tested using the χ^2 test (http://ihg.gsf.de/cgi-bin/hw/hwal.pl); p value < 0.05 was accepted as statistically significant. Genetic distances were estimated by calculating the coancestor coefficient of Reynolds et al. [23].

4. Results

The frequencies of the *AGT* c.704T>C, *MTHFR* c.677C>T, *GPIIIa* c.98C>T and *GSTP1* c.313A>G polymorphisms were analyzed in different Indian and Mestizo-Mexican populations. Distribution of allelic frequencies and comparison of these with other populations are shown in Table 2. The most distinguishing group among the six Mexican populations was the Trikis, given that this group presented a significant genetic differentiation in most of the studied SNPs ($p <$ 0.0002) when compared to the other studied populations; the only exception was *GSTP1*.

Among the most remarkable differences was that observed for the *MTHFR* c.677C>T polymorphism where the Trikis had a very low frequency of the allele C (7.8%), whereas the rank was 29.3% and 53.7% for Purépechas and Yaquis, respectively. Considering the

Table 3
Genotype frequency, heterozygosity (H) and Hardy-Weinberg equilibrium test[a] of four SNPs from six Mexican populations

		MTHFR			*AGT*			*GPIIIa*			*GSTP1*		
Population		CC	CT	TT	CC	CT	TT	CC	CT	TT	AA	AG	GG
Mestizo	n	17	33	21	42	26	3	65	6	0	13	33	25
($n = 71$)	H (%)	*He* = 49.8; *Ho* = 46.5			*He* = 35.0; *Ho* = 36.6			*He* = 8.1; *Ho* = 8.4			*He* = 48.6; *Ho* = 46.5		
Maya	n	7	28	19	42	11	1	51	3	0	6	29	19
($n = 54$)	H (%)	*He* = 47.6; *Ho* = 51.8			*He* = 21.2; *Ho* = 20.4			*He* = 5.4; *Ho* = 5.5			*He* = 47.1; *Ho* = 53.7		
Trikis	n	0	14	75	86	3	0	89	0	0	14	25	50
($n = 89$)	H (%)	*He* = 14.6; *Ho* = 15.7			*He* = 3.3; *Ho* = 3.4			*He* = 0; *Ho* = 0			*He = 42.0; Ho = 28.1*[b]		
Purépechas	n	2	13	14	22	7	0	26	3	0	4	16	9
($n = 29$)	H (%)	*He* = 41.5; *Ho* = 44.8			*He* = 21.3; *Ho*= 24.1			*He* = 9.8; *Ho*= 10.3			*He* = 48.5; *Ho* = 55.2		
Yaquis	n	9	11	7	16	9	2	22	5	0	3	13	11
($n = 27$)	H (%)	*He* = 49.7; *Ho* = 40.7			*He* = 37.0; *Ho* = 33.3			*He* = 17.0; *Ho*= 18.5			*He* = 45.6; *Ho* = 48.1		
Huicholes	n	3	8	4	7	8	0	15	0	0	1	2	12
($n = 15$)	H (%)	*He* = 49.8; *Ho* = 53.3			*He* = 40.0; *Ho* = 53.3			*He* = 0; *Ho* = 0			*He* = 23.1; *Ho* = 13.3		

[a]Heterozygosity expected (He), Heterozigosity observed (Ho) and Hardy-Weinberg equilibrium test.
[b]Significant Hardy-Weinberg dysequilibrium by Pearson's goodness-of-fit χ^2 test (1 df).
SNP, single nucleotide polymorphism.

Table 4
Coancestry coefficent (above diagonal) and combined probability of pairwise comparisons[a] (below diagonal) between Mexican populations and three worldwide racial groups

	Mestizo	Maya	Triki	Huichol	Purépecha	Yaqui	Caucasian	Asian	Blacks
Mestizo	*****	0.0064	0.19114	0.0358	0.0158	−0.0088	0.0780	0.1380	0.1661
Maya	0.1814	*****	0.1194	0.0476	−0.0081	0.0131	0.1436	0.1807	0.2388
Triki	0.0000	0.0000	*****	0.2748	0.0740	0.2694	0.4056	0.4110	0.6021
Huichol	0.1123	0.0556	0.0000	*****	0.0568	−0.0066	0.1639	0.3478	0.2881
Purépecha	0.1257	0.7341	0.0000	0.0133	*****	0.0227	0.1534	0.1834	0.2879
Yaqui	0.5707	0.0637	0.0000	0.1750	0.0510	*****	0.0597	0.1743	0.1304
Caucasian	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	*****	0.0910	0.0723
Asian	0.0000	0.0000	0.0000	0.0029	0.0000	0.0000	0.0000	*****	0.1672
Blacks	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	*****

[a]Exact test: 10,000 permutations.

Bonferroni correction factor, to establish pair comparison significance (Table 2; $p < 0.003$), the other genetically distinctive group was the Mestizo, which was differentiated from the Mayas, Purépechas and Yaquis.

Comparing allelic distribution in the six Mexican ethnic groups with those reported previously in other populations (Table 2), we observed in the case of the *MTHFR* a significant reduction in the frequency of allele C in all studied populations when compared with Caucasian, Asian and Black populations. Moreover, the frequency of *AGT* 704C allele in the Mestizo, Maya, Purépecha, Huichol and Yaqui groups was high when compared to the Caucasian population. Concerning the Trikis, the same allele showed an increased frequency in relation not only to the Caucasian population but also to the Asian and Black populations.

Frequency of *GPIIIa* 98T allele was slightly increased in Mestizo and indigenous Mexican groups when compared with Caucasian and Black populations. On the other hand, the frequency of the allele was similar between the Mexican population and an Asian population.

In regard to the *GSTP1* 313G allele, all Mexican populations (ranking from 14% to 41%) had a reduction in frequency when compared with Caucasian (64%), Asian (83%) and Black (58%) populations.

Distribution of genotypes for all analyzed genetic markers was in Hardy-Weinberg equilibrium (Table 3) with the exception of the distribution observed for *GSTP1* polymorphism in the Trikis. In this ethnic group there was an increment in the homozygous proportion, suggesting a significant endogamous process, which is supported by comparison of the heterozygosis in all the studied Mexican populations. In this respect, the Trikis presented the lowest frequency of heterozygosity in most of the genetic markers as compared with the other groups, with the exception of the *GPIIIa* polymorphism.

Genetic distances were estimated by calculating the coancestor coefficient of Reynolds et al. [23] among the different populations (Table 4). The neighbor-joining tree (Fig. 2) was obtained by using the genetic distances of the Mexican populations and three other racial

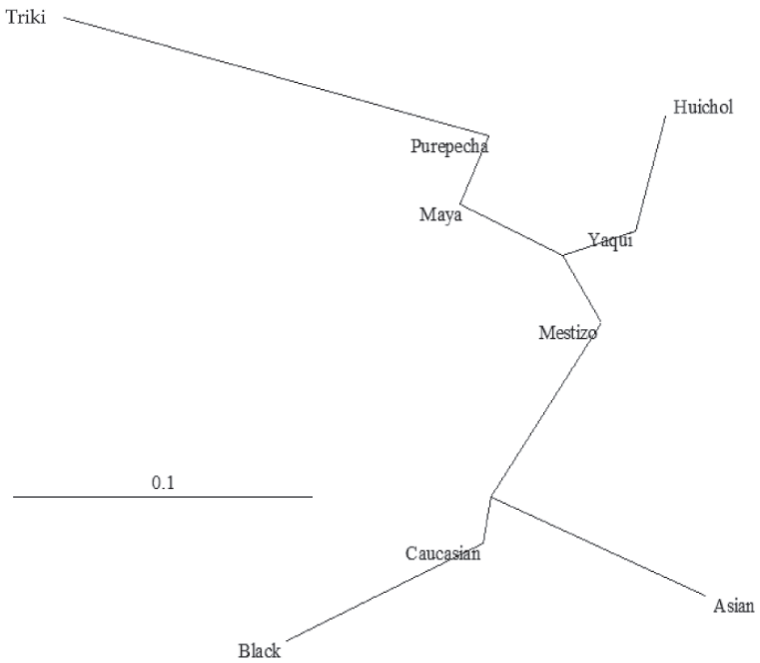

Fig. 2. Neighbor-joining tree using concenstry coefficient distances between Mexican populations and three racial groups based on four SNPs (*MTHFR, AGT, GPIIIa and GSTP1*).

groups (Caucasian, Asian and Black) and graphically demonstrates the relationship among the populations. As suggested for the preceding analysis, Trikis were the most genetically differentiated group.

5. Discussion

The Mexican population has a historical distinction for its ethnic diversity, and the country presents one of the highest concentrations of indigenous populations on the entire American continent (obtained from the Instituto Nacional Indigenista) [24]. According to the most recent population census, the indigenous population of Mexico is 8.7 million (obtained from the Instituto Nacional de Estadística Geofrafía e Informática) [21]. These indigenous subjects are descendants of Amerindian populations with an ancient Asian origin [25]. In addition, the Mexican Mestizos population appears to be the result of the genetic admixture among Amerindians, Caucasian and Black genes. The general pattern has a high Indian ancestry followed by Caucasian and Black ancestry [18]. Because it has been determined that genetic polymorphisms have an ancient origin and are usually found in most human populations but often with different allele frequencies [17], it would be very probable that Mexican populations may have diverse types and frequencies of genetic polymorphisms of different genes that predispose to cardiovascular diseases.

We analyzed the allelic and genotype frequencies in polymorphisms of hypertension, thrombophilic and oxidative stress-related genes *MTHFR*, *AGT*, *GPIIIa*, and *GSTP1* in different Mexican populations. Regarding the *MTHFR* c.677C>T polymorphism, we observed an allelic frequency ranking from 46% to 92%. In general, these frequencies were higher than that found in the Caucasian [26], Asian [27] and Black [28] populations. The elevated frequency of the 677T allele in Mexican populations has also been observed in earlier studies [29,30]. It is noteworthy that our results determined that the Triki population has the highest frequency (92%) of the mutant allele with no homozygous individuals for the 677C allele. In a previous study [30], the frequency of the 677T allele in the Purépecha population was lower (57%) than observed in this study (70%). This difference may be caused by some genetic admixture of the former indigenous population with Caucasian genes.

It has been observed that homozygosity for the 677T allele results in reduced MTHFR enzyme activity and, subsequently, elevated homocysteine concentrations of ∼20% [11], which represents a risk factor for ischemic vascular disease [10]. Likewise, several studies de-

scribed the association between the *MTHFR* 677TT homozygous genotype and an increased risk of hypertension [31], macrovascular abnormalities in systemic sclerosis patients [32], intima-media thickening in patients with cognitive impairment [33], and preeclampsia [34]. However, in a study carried out by our group, it was found that the 677TT genotype confers a reduced risk of preeclampsia in Maya-Mestizo women under a recessive model for the 677T allele [35]. The inconsistency of the results in different populations may be caused by the differential phenotypic expression of specific genotypes as a consequence of diverse factors such as genetic background, age, gender, physiological and pathological conditions, intake of food and drugs, and physical activity [36,37]. All of these findings demonstrate the importance of studying the allelic and genotype frequency of different polymorphisms in diverse human groups.

The *AGT* 704TT genotype increases plasma angiotensinogen levels that, in turn, augment the risk of hypertension [9]. In this study, *AGT* 704C allele and genotype frequencies distribution were similar among Mexicans. In all populations the 704C allele was predominant, and Trikis bear the highest frequencies of this (98%). In contrast, the 704TT genotype was absent in Trikis, Purépechas and Huicholes and was very low in Mestizo, Maya and Yaquis. Comparison of the allele frequencies of the Mexican populations with other previously described allele frequencies in different human groups resulted in being similar between Mexicans and Asians [38] as well as Blacks [39]. In contrast, the 704T allele was lower than that observed in the Caucasian group [40].

Concerning the c.98C>T polymorphism of the *GPIIIa* gene, it seems to predispose premature acute coronary syndrome and stroke in young Caucasian women [14]. The frequency of the mutant allele (98T) was very low in all the studied Mexican populations and was similar to that reported for an Asian [41] and Black population [42]. In contrast, a Caucasian group has the highest presence of the mutant allele with a frequency of 17% [14]. To the best of our knowledge, this is the first report of this polymorphism in a Mexican population.

In regard to the *GSTP1* gene, the 313G allele was present in a higher frequency in Mexicans as compared with Caucasians [43], Asians [44], and Blacks [45]. Currently there are no studies of this genetic marker with other Amerindian populations. In contrast, Hatagima et al. [46] studied the same polymorphism in a Brazilian Mestizo population, finding that the frequency of the 313G allele was 38%, similar to that observed in Caucasian and Black populations. This may be attributed to the fact that the genetic constitution of Brazilians has a high influence over these human groups.

Interestingly, the neighbor-joining tree using coancenstry coefficient distances between Mexican populations and three racial groups showed that Trikis were the most genetically differentiated group. Consequently, this population presented the highest relative genetic homogeneity, a characteristic that may be a consequence of the conservation of its endogamous reproductive cultural patterns [47].

In summary, according to the study of polymorphisms involved in hypertension, thrombophilia and oxidative stress (*AGT*, *GPIIIa*, *MTHFR* and *GSTP1*), we determined their allele and genotype frequencies in different Mexican populations with diverse geographical, anthropological, genetic, and cultural antecedents. Because these polymorphisms have been associated with distinct cardiovascular pathologies, we are currently studying whether the presence of some alleles may be a risk factor associated with these types of diseases in the studied Mexican populations.

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