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Research Report

Novel description of aldosterone synthase *CYP11B2* -344 T>C gene polymorphism related to hypertension in Mexican Amerindians: Teenek, Mixtec and Mayans.

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Abstract – This is a report on population genetics of -344 T>C polymorphism in Amerindians for the first time. The aim was to establish the frequency of this polymorphism present in the 5' promoter region of the *CYP11B2* gene in Amerindian (Mayans, Mixtecos, and Teenek) and Mestizo populations from Mexico. It has been associated with an aldosterone increase and hypertension. Analyses were carried out by 5' exonuclease TaqMan genotyping assays in a group of 608 unrelated individuals belonging to four different Mexican populations (301 Mexican Mestizos, 106 Mayans, 151 Mixtecos, and 50 Teenek). The frequencies in these populations were compared to those reported in other ethnic groups. Results showed significant highest frequencies of *-344 T* allele in Mexican populations (Mestizos and Amerindians) when compared to Europeans and Asians ($p < 0.05$). This confirms uniqueness of Amerindian genetic profile. In summary, the distribution of *-344 T* allele of the *CYP11B2 -344 T>C* polymorphism distinguishes the Mexican population form others, including Europeans and Asians. The knowledge of the distribution of this allele could be useful in Mexican Amerindians for hypertension prevention, epidemiology and anthropology.

Keywords **-** Amerindians; Aldosterone; Renin; Angiotensin; Cytochrome P450; Mexican; Genetics; Teneek; Mixtec; Mayans; Hypertension; Obesity; Metabolic Syndrome; Diabetes

Introduction

The renin-angiotensin-aldosterone system (RAAS) plays an essential role in blood pressure control through regulating fluid and electrolyte balance. Aldosterone plays an important role in electrolyte homeostasis by acting on mineralocorticoid receptor in the cortical collecting ducts of the kidney to increase expression of sodium channels, leading to sodium and water reabsorption and potassium excretion (Brenner et al. 2005; Yan et al. 2012, Conell et al. 2008; Matsubara et al. 2004). Excess production of aldosterone induces sodium and water retention, resulting in increased peripheral vascular resistance and suppression of the renin-angiotensin system (Brenner et al. 2005; Yan et al. 2012, Conell et al. 2008; Matsubara et al. 2004). The aldosterone synthase is an enzyme that has an important role in the conversion of 11-deoxycorticosterone (DOC) to aldosterone that involves three consecutive reactions, an 18-hydroxylation step providing 18-hydroxycorticosterone and, finally, 18-methyloxidation to produce aldosterone, the principal human mineralocorticoid produced in the adrenal gland (Conell et al. 2008).

Aldosterone synthase is encoded by for *CYP11B2* gene that is located in human chromosome 8q22 (Kupari et al. 1998).*CYP11B2*gene presents one important single nucleotide polymorphism (SNP) in the5' promoter region [*-344 T>C* with number of the ID: rs1799998]. This SNP is the most widely studied, because alters a putative recognition site for the transcription factor SF-1 (steroidogenic factor-1) (Basset et al. 2002), and has been associated with risk of developing subclinical atherosclerosis, nephropathy, ischemic stroke, atrial fibrillation, heart failure, hypertension, blood pressure and coronary artery disease (Matsubara et al. 2004; Kupari et al. 1998; Tsukada et al. 2002; Huang et al. 2010; Chen et al. 2011; Borzyskowska et al. 2012; Androulakis et al. 2013, Chen et al. 2011).Several reports have demonstrated large ethnic differences in the allele frequencies of this polymorphism (Huang et al. 2010; Huatanen et al. 1999; Tiret et al. 2000; Russo et al. 2002; Kumar et al. 2003; Barbato et al. 2004; Ganapathipillai et al. 2005; Freitas et al. 2007; Lee et al. 2009; Rajan et al. 2010).

Considering the important role of this polymorphism in the genetic susceptibility to hypertension and the high variability of their frequencies in different populations, the aim of the present work was to analyze the frequencies of this polymorphism in healthy Amerindian and Mestizo populations from Mexico (Fig 1).

Material and Methods

The study included 608 unrelated healthy individuals belonging to four Mexican populations (Mestizos, Mayans, Mixtecos, and Teenek). Healthy parameters were only fully controlled in Mexican Mestizo. A panel of 301 unrelated Mexican Mestizo individuals living in Mexico City was studied. Individuals were asked about their birthplace, as well as that of their parents and maternal and paternal grandparents. We considered as Mexican Mestizo only those who for two generations, including their own, had been born in Mexico (Fig 1). A Mexican Mestizo is defined as someone born in Mexico, who is descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards, of Caucasian and/or sub-Saharan African origin, who came to the American Continent during the 16^{th} century.

On the other hand, 106 unrelated individuals from Maya ethnic group belonging to the Macro-Maya linguistics were included in the study. The samples were obtained in a village of Merida in the Yucatán State of Mexico (Fig 1). A group of 151 unrelated individuals from Mixteco ethnic group belonging to the Macro-Mixteco linguistic family were also studied. In this case, the samples were obtained in four communities located in Jamiltepec, Oaxaca State of Mexico (Fig 1). Finally, 50 unrelated individuals belonging to a group of linguistically unclassified people (Teenek) were analyzed. The samples of these individuals were obtained in the San Vicente Tancuayalab village in the Huasteca region of San Luis Potosi State in Mexico (Fig 1) (Cavalli-Sforza et al. 1997; Etnografia de los pueblos indigenas de Mexico, 1995 a, 1995b). Ascents of indigenous individuals had lived in the same place for at least three generations.

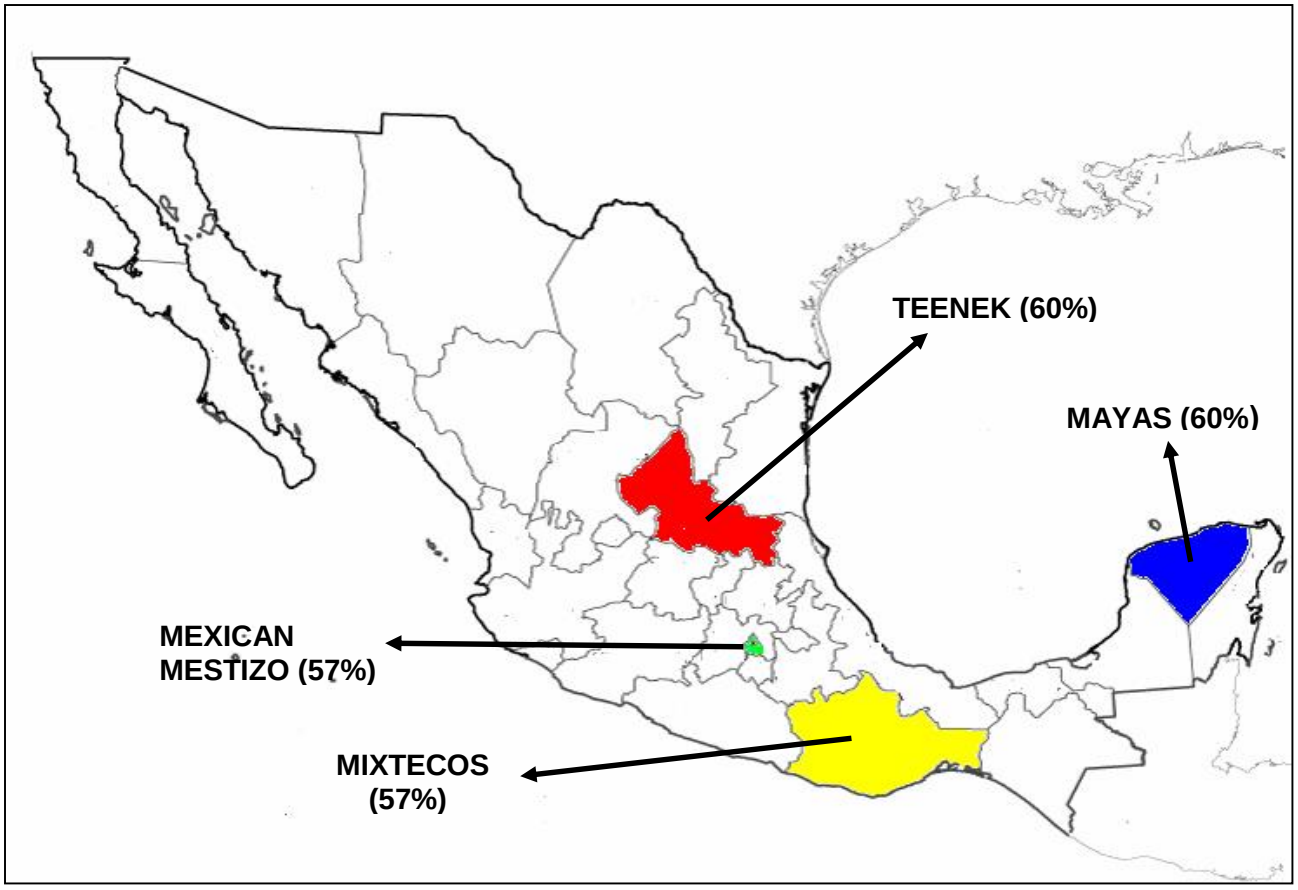

Fig. 1. Map of the Mexican Republic showing populations of the study (yellow Oaxaca State, blue Yucatan State, red San Luis Potosi, and Green Mexico City).
The (%) indicate the distribution of the (*-344 T)* allele in the different populations in study.

DNA methodology proposed by Lahiri and Nurnberger was used for DNA extraction (Lahiri and Nurnberger, 1991). The genotyping of *CYP11B2* -344T>C (rs1799998) single nucleotide polymorphism, was carried out by TaqMan® genotyping assays as per manufacture's protocol (Applied Biosystems, USA). The single nucleotide polymorphism was amplified using thermocycling conditions as follows: Initial denaturation at 95°C for 10 min followed by 40 cycles of 15 sec at 95°C (denaturation) and 1 min at 60°C (annealing/extension). Thermal cycling in 7900 HT Real-Time polymerase chain reaction (PCR) Systems and data analysis was performed by the SDS3.1 Software for Applied Biosystems, USA.

Allele and genotype frequencies of the *CYP11B2* gene polymorphism was obtained by direct counting. Also, Hardy–Weinberg equilibrium (HWE) was calculated using the chi-square test. Allele frequencies obtained in Mexican populations were compared among them and with those reported in other populations using Mantel-Haenszel's chi-square. Fisher's exact test was used if the number in any cell of the 2 × 2 contingency table was less than 5. The statistical program EPIINFO 7.0 (USD Incorporated, Stone Mountain, GA) was used for these analyses. The groups used for comparisons included populations from Caucasian, Asian, African, and American origin.

Results

Allele and genotype frequencies of the single nucleotide polymorphism of the *CYP11B2* gene studied in the four Mexican populations are summarized in Table 1.

Table 1. Allele (af) and Genotype (gf) frequencies of CYP11B2 gene (CYP11B2 -344 *T>C*) polymorphism in Mexican Mestizos and Amerindian populations.

	Mexican Mestizos (n=301)		Mayans (n=106)		Mixtecos (n=151)		Teeneks (n=50)	
rs1799998 CYP11B2* (-344 *T>C*)								
Allele	n	af	n	af	n	af	n	af
C	258	0.428	85	0.400	130	0.430	40	0.400
T	344	0.571	127	0.599	172	0.569	60	0.599
Genotype	n	gf	n	gf	n	gf	n	gf
CC	57	0.189	17	0.160	32	0.211	8	0.160
CT	144	0.478	51	0.481	66	0.437	24	0.480
TT	100	0.332	38	0.358	53	0.350	18	0.360

All populations were in Hardy-Weinberg equilibrium. n = number; af = Allele frequency; gf = gene frequency.
* The genetic distribution of the CYP11B2 -344 *T>C* (rs1799998) polymorphism in our study groups were similar among Mexican Mestizos, Mayas, Mixtecos, and Teeneks.

The observed and expected frequencies of the polymorphic site were in Hardy-Weinberg equilibrium. Similar distribution of the polymorphism was observed in the Mexican populations (Mestizos and Amerindians). In addition, the allele and genotype frequencies of the -344 T>C polymorphism in the four Mexican populations were compared to those reported in other populations, such as European, Australian, Asian, and African (Table 2). The -344 *T* allele frequency was higher in Mexican populations than in Europeans and Australians. On the other hand, this frequency was lower in

Mexicans when compared to Asians (Koreans and Chinese) and African. Similar distribution of *-344 T* allele was observed in Mexicans as compared to Brazil admixed population (Freitas et al. 2007).

Table 2. Allele frequencies (%) of cytochrome *P45011B2* (*CYP11B2 -344T>C*) polymorphism in different populations

Population	n	*T*	*C*	Reference
Mexican Populations*				
Mestizo	301	57 [a]	43	Present study
Mayans	106	60 [b]	40	Present study
Mixtecos	151	57 [c]	43	Present study
Teenek	50	60 [d]	40	Present study
Caucasian populations				
Australian	291	42	58	[Kumar et al. 2003]
Finnish	270	47	53	[Huatanen et al. 1999]
French	393	54 [e]	46	[Tiret et al. 2000]
German	163	45	55	[Brand et al. 1999]
Italian	811	52 [f]	48	[Russo et al. 2002]
Asian populations				
Chinese (Shanghai)	120	67.5	32.5	[Huang et al. 2010]
Korean	134	68	32	[Lee et al. 2009]
Amerindian populations*				
Brazil (NWA)	78	59	41	[Freitas et al. 2007]
Venezuela	91	56	44	[Ganapathipillai et al. 2005]
Africans (UK)	441	449	79	21 [Barbato et al. 2004]

Abbreviations: UK= United Kingdom; NWA= Northwest Amazonas; *p*= p-value.

[a, b, c, d] Significantly increased frequencies of the *T* allele in Mexican populations when compared to Caucasian populations [Finnish ($p=0.0004$, $p=0.001$, $p=0.004$ and $p=0.01$, respectively), German ($p=0.0002$, $p=0.0004$, $p=0.001$ and $p=0.006$, respectively), and Australian ($p=2x10^{-6}$, $p=8x10^{5}$, $p=2x10^{-4}$ and $p=0.0008$, respectively)].

[a, b, c, d] Significantly decreased frequencies of *T* allele in Mexican populations when compared to Africans ($p=1x10^{-8}$, $p=1x10^{-7}$, $p=1x10^{-7}$ and $p=1x10^{-7}$, respectively).

[a, c] Decreased frequencies of the *T* allele in Mexican populations (Mestizos and Mixtecos) when compared to Asians populations [Chinese ($p=0.005$) and Korean ($p=0.01$), respectively].

[b, d] The distribution of this polymorphism was similar in Mexican (Mayans and Teeneks) and Asian populations (Chinese and Korean).

[e, f] The distribution of this polymorphism was similar in Caucasian (French and Italian) and Mexican populations.

*, ** The distribution of this polymorphism was similar in Mexican and Amerindian populations.

Discussion

This is the first published study of *CYP11B2* -344 T>C in Amerindian comparative populations genetics. Brazil and Venezuela studies were carried out in admixed populations (Table 2). It describes the allele and genotype frequencies of the *CYP11B2* -344 T>C polymorphism of the *CYP11B2* gene in Mexican Mestizos and three Mexican Amerindian populations (Mayas, Mixtecos, and Teenek) belonging to different linguistic trunks (Cavalli-Sforza et al. 1997; Etnografia de los pueblos indigenas de Mexico. 1995a, 1995b). Mexican populations (Mestizos and Amerindians) presented similar distribution of the studied polymorphism. Teenek, Mayan, and Mixteco populations have been studied previously using other different polymorphisms (Gomez-Casado et al. 2003; Vargas-Alarcon et al. 2006). In addition, Arnaiz-Villena et al., report the genetic separation of Amerindian populations (including Teenek, Mayan, and Mixteco) from other Worldwide populations by using HLA gene studies (Arnaiz-Villena et al. 2000; Arnaiz-Villena et al. 2010; Arnaiz-Villena et al. 2014).

These studies reported that Amerindian populations show important differences with worldwide ones. We verified these differences using a different polymorphism (*CYP11B2* -344 *T>C*) not previously studied on these populations. Frequencies of the *CYP11B2* -344 *T>C* polymorphism in Mexican populations were compared to those reported in other populations. The comparison showed that Mexican Mestizos, Mayas, Mixtecos, and Teeneks present a higher frequency of the -344 *T* allele (57%, 60%, 57%, and 60%, respectively), and a lower frequency of the -344 *C* allele (43%, 40%, 43%, and 40%, respectively) when compared to European and Australian populations; sub-Saharan Africans bear the highest T allele frequency. On the other hand, Mexicans presented a lower frequency of the -344 *T* allele when compared to Asian populations (Korean and Chinese populations). The present study is the first in Amerindian populations and showed a high frequency of the-*344 T* allele in these populations. The *CYP11B2* -344 *T>C* polymorphism is the most widely studied, as it is important for binding of the steroidogenic factor-1, the transcriptional regulatory protein (Basset et al. 2002). This polymorphism either increases the aldosterone to renin ratio (ARR) in essential hypertension or decreases aldosterone production, leading to sodium wasting and decreased excretion of potassium (Matsubara et al. 2004). Several studies have associated

one or other (T or C) -344 *T>C* polymorphism with hypertension and other cardiovascular pathologies like hypertension and myocardial infarction (Matsubara et al. 2004; Kupari et al. 1998; Tsukada et al. 2002; Huang et al. 2010; Chen et al. 2011; Borzyskowska et al. 2012; Androulakis et al. 2013; Brand et al. 1999). These studies were conducted extensively in populations of Caucasian, and Asian origin (Huang et al. 2010; Huatanen et al. 1999; Tiret et al. 2000; Russo et al. 2002; Kumar et al. 2003; Barbato et al. 2004; Ganapathipillai et al. 2005; Freitas et al. 2007; Lee et al. 2009; Rajan et al. 2010; Brand et al. 1999). Nonetheless, studies in Amerindians do not exist. The present data showed high frequencies of the -344 *T* allele in Mexican populations. This data clinical significance is yet unclear since studies on relationship to pathology are lacking in studied populations and are now being accomplished. In summary, the present study establishes the high frequency of *CYP11B2* - 344 *T* allele in Mexican populations (Mestizos and Amerindians), and distinguishes these populations from other groups of European, African, and Asian origin. Finally, *CYP11B2 –344 T and / or C* allele is known to be associated with development of cardiovascular diseases in different populations and knowledge of this allele distribution in our Mexican populations could be helpful to understanding this genetic susceptibility marker role.

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Conflicts of Interest

There are not competing financial interests in this study.
The authors alone are responsible for the content and writing of the paper.

Abbreviations

***RAAS*:** Renin-angiotensin-aldosterone system
DOC: Deoxycorticosterone
CYP11B2: Cytochrome P450, family 11, subfamily B, polypeptide 2
SNP: Single nucleotide polymorphism
SF-1: Steroidogenic factor-1
HLA: Human leukocyte antigen

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