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Polymorphisms of *APLN-APLNR* system are associated with essential hypertension in Mexican-Mestizo individuals

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ABSTRACT

The aim of this study was to evaluate if polymorphisms of APLN and APLNR genes may play a role as susceptibility markers for hypertension in a group of Mexican-Mestizo patients. A case-control study was carried out including normotensive and hypertensive individuals. For these, two polymorphisms of APLN (rs3761581 and rs56204867) and two of APLNR () genes were genotyped by 5′ exonuclease TaqMan assay in 400 normotensive individuals and 383 patients. The results showed that, under an additive model adjusted by BMI, HDL, triglycerides, glucose and family history of essential hypertension, the rs7119375 and rs10501367 polymorphisms of APLNR gene were associated significantly with a decreased risk of essential hypertension ($P = 0.039$ and $P = 0.029$, respectively). Besides, the haplotypes analysis of these polymorphisms showed that H1 haplotype was associated with an increased risk of essential hypertension ($P = 0.026$), while the H2 haplotype was associated with a decreased risk ($P = 0.032$). Contrary, the rs3761581 and rs56204867 polymorphisms of APLN gene were not associated with essential hypertension ($P = 0.1707$ and $P = 0.0769$, respectively). The data suggest that APLNR rs7119375 and rs10501367 are associated with a decreased risk of essential hypertension in our Mexican-Mestizo studied group, but further studies are warranted.

1. Introduction

Hypertension is a main public health problem, which according to a global study will affect 1.56 billion adults by the year 2025 (Lim et al., 2012). In regard to México, the last National Survey performed in 2012, demonstrated that the prevalence of hypertension in adults was of 31.5%, being more frequent in men than in woman (33.3% *versus* 30.8%, respectively) (Instituto Nacional de Salud Pública, 2012).

Essential hypertension (EH) is a multifactorial complex disease characterized by a sustained elevation in blood pressure with no known underlying medical or biological cause, in which genetic determinants, environmental factors and their interactions playing roles in the development of the disease (Lifton et al., 2001).

Twin and family studies aimed to approach EH heritability suggest that approximately 30–60% of blood pressure variation is determined by genetic factors (Kupper et al., 2006; Shih and O'Connor, 2008). Despite this, EH remains as a complex phenotype in which genetic determinants remain largely undefined (Doris, 2011). A recent large study of Sardinian families estimated a broad-sense heritability of ~65% and ~45% for systolic and diastolic blood pressure, respectively (Pilia et al., 2006). In general, large-scale epidemiologic studies suggest that the

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heritability of blood pressure exceeds 50%, justifying a search for genetic variants influencing susceptibility to EH (Levy et al., 2000; Lifton et al., 2001).

Several candidate genes have been investigated in relation to hypertension. The apelin (APLN) and its receptor APLNR (APLN-APLNR system), is a peptide signaling pathway which play a role in cardiovascular homeostasis (Reaux et al., 2001). Studies in animals suggest that this system is involved in the regulation in volume homeostasis and blood pressure (Kagiyama et al., 2005; Charles, 2007). Besides, several genetic variants of the genes that codify for the proteins of this signaling pathway have been examined as susceptibility markers for hypertension. Regarding, it has been found that rs3761581: T>G and rs56204867: G>A of *APLN*, rs7119375: A>G and rs10501367: T>C of *APLNR* were associated with EH in individuals of Han Chinese population (Li et al., 2009; Niu et al., 2010; Li et al., 2015; Liu et al., 2014).

Taking into account the important ethnic differences that have been observed in the risk of developing hypertension, and the relevance of the APLN-APLNR signaling pathway in the cardiovascular homeostasis, the aim of this study was to analyze whether *APLN* and *APLNR* polymorphisms were associated to the risk of present EH in a group of Mexican-Mestizo patients.

2. Subjects and methods

2.1. Subjects

Institute's Human Research Committees approved the study. Informed consent was obtained from all individuals before participation in the study. A case-control study was performed. Three hundred eighty three individuals with essential hypertension and 400 normotensive individuals (controls) were analyzed; all were of Mexican-Mestizo ethnic origin. Only individuals born in México and with a Spanish-derived last name along with a family of Mexican ancestors dating back to the third generation were considered Mexican-Mestizo (Gamboa et al., 2000).

The case group was recruited from the National Institute of Cardiology (INCICH) in Mexico City, México; hypertension was defined as systolic blood pressure (BP) ≥ 140 mm Hg, diastolic BP ≥90 mm Hg or the use of at least one class of antihypertensive drugs. Secondary hypertension was ruled out using a detailed health questionnaire and clinical evaluation. None of the patients had evidence of cardiac or renal failure. The control group was recruited among blood donors of the same Institute, they had systolic and diastolic blood pressures <120 and <80 mm Hg, respectively, and were not taking any antihypertensive medication. Furthermore, all individuals were evaluated for various clinical characteristics as previously reported (Martínez-Rodríguez et al., 2013).

2.2. Methods

2.2.1. Genotyping and quality control

Genomic DNA was isolated from peripheral blood using a commercial kit based on the salt fractionation method (QIAmp 96 DNA Blood Kit, Quiagen, Hilden, Germany). Polymorphisms studied were rs56204867 and rs3761581 of *APLN*, rs7119375 and rs10501367 of *APLNR*.

Real-time PCR allelic discrimination TaqMan assay (Thermo Fisher Scientific, Waltham, MA, USA) was used for genotyping analysis. All PCR reactions contained 10 ng of DNA, 5.0 μL TaqMan Universal Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) (2×), 0.25 μL primers and probes (10×), as well as water, for a final volume of 10 μL, including the appropriate negative controls in all assays. For all polymorphisms, the assay used probes and primers designed by assay-on-demand services from Applied Biosystems: for rs3761581, assay ID: C__27476888_10 and forrs56204867, assay ID: C__89641838_10, both of *APLN*; for rs7119375, assay ID: C__27852601_10 and for rs10501367, assay ID: C__30176584_10, both of *APLNR*.

Real-time PCR was performed on a LightCycler® 480 Instrument (Roche Diagnostics Ltd., Switzerland). Conditions for all polymorphisms were 95 °C for 10 min, and 40 cycles of amplification (95 °C for 15 s and 60 °C for 1.30 min). For each cycle, the software determined the fluorescent signal from the VIC- or FAM-labeled probe. Genotyping call rate surpassed 95% for all SNPs tested, with no discordant genotypes in 10% of duplicate samples.

2.2.2. Statistical analysis

Demographic and clinical variables between hypertensive and non-hypertensive groups were analyzed using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA). Numeric variables not normally distributed are presented as median and range and were compared using the Mann–Whitney *U* test. Comparisons between categorical variables and deviations from Hardy–Weinberg equilibrium (HWE) were carried out using χ^2 test. Normally distributed numeric variables are presented as mean ± SD and compared using the Student's *t*-test.

A logistic regression analysis for *APLNR* and Fisher's exact test for *APLN* polymorphisms were used to test the associations between genotype and hypertension under additive, dominant and recessive inheritance models. A logistic regression analysis was used to test the associations between genotype and hypertension under additive, dominant and recessive inheritance models, reporting the most significant. Odds ratio (OR) and P-values were adjusted by age, sex, body mass index (BMI), glucose, triglycerides, high-density lipoprotein (HDL) and family history of essential hypertension.

Pairwise linkage disequilibrium (LD, r^2) estimations between polymorphisms and haplotype reconstruction were performed with Haploview version 4:1, and haplotypes with a frequency < 0.05 were excluded. (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA).

Statistical power to detect an association of four polymorphisms analyzed with hypertension at an alpha of 0.05 was calculated taking into account the frequencies of the polymorphisms and the prevalence of hypertension in adult population (Campos-Nonato et al., 2013), under an additive model using QUANTO software (http://hydra.usc.edu/GxE/).

3. Results

The clinical and biochemical characteristics of the individuals with EH and controls are shown in Table 1. Except for total cholesterol and low-density lipoprotein, the individuals with EH showed significant differences of all parameter in comparison with the control group.

Hardy-Weinberg equilibrium test was performed for the polymorphisms under study. Considering that, the *APLN* is located on X chromosome (Xq25-q26.3), the HWE test of rs3761581 and rs56204867 was only performed in females. The distribution of the observed genotypes did not either differ from what was expected in the patient or control groups (P > 0.05).

Allele and genotype frequencies of rs3761581 and rs56204867of the *APLN* are shown in Table 2. We did not find association of these polymorphisms with essential hypertension (rs3761581: P = 0.1707; rs56204867: P = 0.0769).

Regarding to rs7119375 and rs10501367 polymorphisms of *APLNR*, both were associated significantly under the additive model with a lower risk of essential hypertension (OR = 0.717; 95% CI: 0.522–0.984; P = 0.039; OR = 0.703; 95% CI: 0.512–0.964; P = 0.029, respectively) after adjusting by BMI, HDL, triglycerides, glucose, and family history of essential hypertension (Table 3). Statistical power of the association of rs13306560 and rs1801133 with hypertension was of 0.82 taking into account a polymorphisms frequency of 0.25, a population risk of 0.3 and an OR of 0.7.

When we analyzed the linked disequilibrium (LD) among the two SNPs of *APLN* and the two of *APLNR* in our population, we observed