CLINICAL INVESTIGATION

The Association of *ADRB1* and *CYP2D6* Polymorphisms With Antihypertensive Effects and Analysis of Their Contribution to Hypertension Risk

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ABSTRACT

Background: Genetic factors have a vital influence on the pathogenesis of hypertension. In this retrospective study, we aimed to evaluate the association of *ADRB1* and *CYP2D6* polymorphisms with antihypertensive effects and perform an analysis of their contribution to hypertension risk.

Methods: A total of 261 healthy individuals and 261 essential hypertension patients treated with metoprolol for 12 weeks were enrolled. *ADRB1* and *CYP2D6* genotypes were identified by xTAG liquid chip technology. We used multivariate logistic regression and a generalized linear mixed model to assess hypertension-related risk factors.

Results: The allele frequencies of *ADRB1* and *CYP2D6* variants were 59.8% and 64.6% in the essential hypertension group and 70.3% and 65.9% in the controls, respectively. The genotype and allele distribution of *ADRB1* were significantly different between the 2 groups ($P < 0.05$), but there was no significant difference in *CYP2D6* distribution ($P = 0.91$ and 0.88). By logistic regression analysis, high fasting plasma glucose, smoking, high triglyceride and the Gly/Gly polymorphism in Arg389Gly *ADRB1* all emerged as independent risk factors for hypertension. Additionally, the *ADRB1* genotype played a major role in the antihypertensive effect of metoprolol and the patients with the Gly389Gly genotype showed a significantly better response to metoprolol than did those with a heterozygous *ADRB1* mutation (Arg389Gly) ($P = 0.027$).

Conclusions: The results demonstrate that Gly/Gly polymorphism in Arg389Gly *ADRB1* was an independent risk factor together with high fasting plasma glucose, smoking and high triglyceride; moreover, the patients who carried the Gly389Gly genotype had a significantly improved metoprolol antihypertensive effect than those with *ADRB1*.

Key Indexing Terms: β1-adrenergic receptor; Cytochrome P450 2D6; Essential hypertension; Risk factor; Metoprolol. **[Am J Med Sci 2017;▮(▮):▮▮▮–▮▮▮.]**

INTRODUCTION

Essential hypertension (EH) is a polygenic disease caused by the combined effects of genetic and environmental factors.[1,2] Despite much investigation, its etiology and pathogenesis are not completely understood. Recently, numerous studies have shown that genetic elements play a pivotal role in the pathogenesis of EH.[3,4] β-blockers regulate the sympathetic response by blocking human β1-adrenergic receptor (*ADRB1*) and are important in the management of EH. *ADRB1* is one of the members of a family of 7 transmembrane G-protein-coupled receptors.[5] *In vitro* studies have revealed that the patients with EH who carried the homozygous *ADRB1* Arg389 variant showed a greater response to β-blocker stimulation than those who carried the Gly389 allele.[6,7] Another hypertension-related gene, *CYP2D6*, may also be important for this; *CYP2D6* polymorphism was reported to affect the metabolic rate of metoprolol in humans.[8,9] However, whether *ADRB1* polymorphisms are correlated with the morbid risk of hypertension remains controversial.[5]

As noted, several studies have focused on either *ADRB1* or *CYP2D6*, but no study of these genes has focused on evaluating the risk of hypertension onset and treatment for patients with hypertension. Here, we aimed to determine the association of *ADRB1* and *CYP2D6* polymorphisms with the risk of suffering from EH and the antihypertensive effect of the β-blocker metoprolol.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Ethics Committee of the First Hospital of Longyan, Fujian (2012002). All patients provided signed informed consent. All the clinical specimens analyzed in this work were collected as part of routine medical care.

Study Subjects

A total of 261 patients with EH who were hospitalized in the First Hospital of Longyan, Fujian and 261 healthy controls were enrolled from July 2012 to December 2015. The cases followed the diagnostic standards of hypertension set by World Health Organization/International Society of Hypertension (WHO/ISH) in February 1999.[10] We excluded patients with comorbidity owing to severe heart, liver or kidney disease, patients for whom administration of other drugs would affect metoprolol treatment and cases with incomplete clinical data or dropout.

For analysis of the unrelated controls, we excluded patients with acute or chronic diseases or a family history of the same as well as patients with abnormal findings in their blood, urine, stool, serum biochemistry, blood stream changes, chest X-ray, heart and abdomen ultrasound or electrocardiograph.

Specimen Collection

The following general clinical information was collected: gender, age, height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), triglyceride (TG) levels, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG) and uric acid (UA) levels.

All the patients received treatment with metoprolol (25 mg twice a day) or metoprolol tartrate sustained-release tablets (40.5 mg once a day) for 12 weeks; blood pressure was measured once every 4 weeks. The treatment was considered to be effective if a decrease of ≥10 mm Hg in systolic blood pressure was found. Before the treatment, 2 mL of blood was collected in an EDTA-Na_2 anticoagulant tube from each patient and was sent to Yi Shan Medical Testing Center of Guangzhou for genotyping.

Definition

High FBG was defined as venous plasma FBG ≥ 6.1 mmol/L. Dyslipidemia was defined as high TC ≥ 5.18 mmol/L, high LDL-C ≥ 3.37 mmol/L, high TG ≥ 1.70 mmol/L and low HDL-C < 1.04 mmol/L. High UA was defined as fasting SUA levels on two different days were above 420 μmol/L (men) or 360 μmol/L (women) under normal state purine diet. Smoking status was defined as one pack a week for more than 6 months.

Genotyping and the xTAG Liquid Chip Technology

Peripheral blood DNA was extracted and then used to detect the genotypes *ADRB1* and *CYP2D6*. The main steps involved in the xTAG liquid chip technology were as follows: (1) Multiplex PCR amplification (including the gene sequence of the mutation site) using the following primers: *ADRB1* wild-type primer, 5′-CCGCAAGGC CTTCCAGG-3′; *ADRB1* mutant primer, 5′-CGCA AGGCCTTCCAGC-3′; *CYP2D6* wild-type primer, 5′-GGGCTGCACGCTACC-3′; *CYP2D6* mutant primer, 5′-GGGCTGCACGCTACT-3′. (2) PCR product digestion by exonuclease enzyme I (*EXO* I) and shrimp alkaline phosphatase (SAP); excess primers and free nucleotides were removed. (3) Multiplex allele-specific primer extension/target specific primer extension was performed, with the right end of the primer connected to the specific sequence of the mutation site in the target gene, and the left end connected to a tag sequence. (4) The allele-specific primer extension reaction product was hybridized with a probe labeled with an anti-tag specific antibody wrapped in polystyrene microspheres. After hybridization, streptavidin-biotin (PE) was added to produce fluorescence; finally, data were obtained on Luminex 200 and analyzed to determine the genotype of each locus in the sample.

Statistical Analysis

All data were analyzed with SPSS 19.0 software and $P < 0.05$ was considered statistically significant. Data are displayed as mean ± standard deviation, median (quartiles) or percentage. Measurement data were compared between groups by the *t* test, Mann-Whitney *U* test or χ^2 test. Multivariate logistic regression and the generalized linear mixed model were used to assess hypertension-related risk factors. Two-factor repeated measures analysis of variance was used to evaluate the association of β-blocker antihypertensive efficacy with *ADRB1* and *CYP2D6* polymorphisms.

RESULTS

Patient Characteristics

The values obtained for age, BMI, SBP, DBP, TG, TC, LDL-C, FBG and UA were significantly higher in hypertensive patients than that in the controls ($P < 0.05$). However, no differences in gender composition or HDL-C ($P > 0.05$, Table 1) were found. The ages of the hypertensive patients were mostly between 40 and 59 years. Patients with grade II hypertension accounted for 81.6%; cases with a disease course ≥1 year accounted for 92.7% of cases (Table 2).

Frequency Distribution of ADRB1 and CYP2D6 Polymorphisms

Of the 261 EH patients studied, 46 (18%) were *Arg/Arg*, 118 (45.2%) were *Arg/Gly* and 97 (37%) were *Gly/Gly ADRB1* genotypes. In the case of *CYP2D6*, 40 (15%) had wild-type *1/*1, 105 (40.2%) the *10/*10 homozygotes and 116 (44.4%) the *1/*10 heterozygotes. As shown in Table 3, minor allele frequencies in the EH and control groups were 59.8% and 70.3% for the *ADRB1* allele and 64.6% and 65.9% for the *CYP2D6* allele, respectively. The genotype and allele distribution of *ADRB1* were significantly different between the 2 groups ($P < 0.05$), but no significant differences were found for