TBXAS1 Gene Polymorphism is Associated with the Risk of Ischemic Stroke of Metabolic Syndrome in a Chinese Han Population

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Abstract

Metabolic syndrome (MS) is caused by genetic and environmental factors, thromboxane A synthase 1 (TBXAS1) gene polymorphism is associated with metabolic disease. This study would explore the relationship between TBXAS1 gene polymorphism and MS. A total of 3072 eligible subjects were obtained, of which 1079 cases were normal controls, 1993 cases were MS patients. subjects were followed up for 5 years and the endpoint events were recorded. The gene polymorphism of TBXAS1 was detected by using Sequenom MassARRAY methods. The results showed that significant differences were observed in ischemic stroke and the genotypes of NC_000007.14:g.139985896C>T (all $P < 0.05$). The incidence of ischemic stroke was significantly higher in T allele carriers than in C allele carriers (all $P < 0.05$). C allele was the protective factor of the onset of ischemic stroke. The interaction effects showed there were negative interactions between C allele and waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, HDL-C and FPG; These findings suggest that NC_000007.14:g.139985896C>T was related to the incidence of ischemic stroke in the whole and MS population, individuals who carry the C allele have a reduced risk of stroke.

Introduction

Metabolic syndrome (MS) is a clustering of some cardiovascular risk factors, including obesity, hypertension, hyperglycemia and dyslipidemia. In recent years, with the effects of an aging population, overeating, lack of exercise and other adverse lifestyles, the prevalence of MS has continued to increase globally. Epidemiological studies in western countries showed that, the prevalence of MS in adults is about 20-30%[1]. In China, a survey showed that the prevalence of MS was approximately 18% -22% [2]. As a chronic lifelong disease, MS is closely related to diabetes and cardiovascular disease. Previous studies have confirmed that MS can increase the risk of diabetes by 5 times [3], increased the risk of cardiovascular events by 2 times [4], and increased all-cause mortality by 1.5 times [5]. In addition, MS is an important risk factor for stroke, which could increase the risk of ischemic stroke by a factor of 2 [6]. Therefore, it is necessary to study the MS and related diseases. Studies have shown that [7], multiple risk factors are related to the onset and prognosis of MS, but even when exposed to the same environment, some populations are still more prone to MS or target organ damage than others. Recent studies have found MS has family aggregation, as well as racial and regional differences[8, 9]. At the same time, the components of MS, such as hypertension, abnormal glucose and lipid metabolism, also have a genetic predisposition [10, 11], which indicate that MS has a significant genetic susceptibility. Therefore, it is currently believed that MS is a complex disease with the combined action of genetic factor and environmental factor, in which genetic factor is internal factor that play an important role in the development of MS. In

recent years, several susceptibility genes related to MS and its components have been identified, such as the retinol-binding protein 4 gene[12, 13], angiopoietin-like protein gene[14], and glucokinase regulatory protein gene[15]. These genes mainly affect the onset and prognosis of MS by affecting the intermediate phenotypes of MS, therefore, by studying the susceptibility genes related to MS, we can not only elucidate the pathogenesis of the disease from the genetic perspective, but also screen the susceptible population at an early stage, providing new ideas and prospects for the comprehensive treatment of MS. Thromboxane synthase (TS) is a microsomal enzyme that belongs to the cytochrome P450 (CYP450) family. TS is listed as CYP5 according to the nomenclature of P450 [16, 17]. The function of TS is to catalyze the isomerization of prostaglandin H2 (PGH2) to thromboxane A2 (TXA2). TXA2 is an effective inducer of platelet aggregation, release and smooth muscle contraction, playing an important role in regulating vascular tension, maintaining blood fluidity and hemostatic mechanism[18]. Lack of platelet TS activity can lead to bleeding, while excess of TXA2 is associated with the pathological process of many diseases, such as cardiovascular diseases [19]. The human thromboxane A synthase 1 (TBXAS1) gene is located on chromosome 7q34[20, 21], including 13 exons and 12 introns[21, 22]. As its important role in thrombotic diseases, it has become a research focus in recent years. Oh et al. reported that the rare allele of NC_000007.14:g.139971318T>A may play a protective role against aspirin hypersensitivity via a lower catalytic activity of the *TBXAS1* gene [23]. Lemaitre et al. studied more than 30 single nucleotide polymorphisms (SNPs) in the *TBXAS1* gene and found that NC_000007.14:g.139954457C>T, NC_000007.14:g.139985896C>T, and NC_000007.14:g.139964799A>C are all related to the onset of myocardial infarction, and NC_000007.14:g.139985896C>T is also associated with ischemic stroke [24]. As the risks of cardiovascular and cerebrovascular disease in MS patients are significantly higher than that in normal people, and the relationship between this gene and MS has not been reported, Whether *TBXAS1* gene is a susceptible gene for MS and its components, or plays a role in MS-related damage needs further exploration and verification. Therefore, we will study the distribution of *TBXAS1* gene SNPs in the Han population in Shandong, China, explore the relationship between *TBXAS1* gene polymorphisms and MS, and provide new ideas for the prevention and treatment of MS.

Materials and Methods

Study population

An epidemiological survey about MS was conducted of 21,700 subjects from January 2007 to December 2007. All participants were from the Han population in China. According to random sampling, a total of 3072 eligible subjects were obtained, of which 1079 cases were normal controls, 1993 cases were patients with MS. From January to December 2012, the study subjects were followed up and the endpoint events were recorded. This study was approved by the ethics committee of Qilu Hospital of Shandong University. Written informed consent was obtained from all subjects. MS was defined according to the joint recommendations of the International Diabetes Federation, American Heart Association, and National Heart, Lung, and Blood Institute [25]. The exclusive criteria were: secondary hypertension, severe heart failure, renal failure or valvular heart disease. Individuals with missing covariates, missing biochemical data, undetected or discordant genotype were also excluded. Endpoints: all-cause death, ischemic stroke, coronary heart disease, myocardial infarction, new-onset diabetes, new-onset hypertension and new-onset MS.

Demographic data collection, clinical and biological assessment

The clinical data were assessed by questionnaire. Height, weight and waist circumference were measured and body mass index (BMI) was calculated. Blood pressure was measured with the use of OMRON HEM-7011 electronic sphygmomanometer (Omron, Japan), SBP and DBP were defined as the average of three readings. Venous blood samples were obtained from all subjects after fasting for at least 12 hours for biochemical determination and DNA extraction.

SNPs selection and genotyping

SNPs were selected based on the following criteria. Firstly, SNPs located within TBXAS1 gene with a MAF of $> 5\%$ in CHB according to the NCBI HapMap Database (http://hapmap.ncbi.nlm.nih.gov/). Secondly, selected SNPs were entered into Haploview Ver. 4.2 software [26] to obtain tag SNPs. Thirdly, no studies

have addressed the distribution regularities of the SNPs, or their relationship with MS, in Chinese population. At last, the SNP rs3801150 was finally selected and genotyped.

Genomic DNAs were extracted from blood by Magen blood DNA kit D3133-03 (Magen, Guangzhou, China) following the manufacturer's protocols. SNP at NC_000007.14:g.139985896C>T was genotyped in BGI, Shenzhen, China by Sequenom MassArray system (Sequenom, San Diego, CA). The PCR reaction was conducted using GeneAmp PCR System 9700 (ABI, Foster City, CA, USA). And mass determination was performed with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry [24]. Data were collected by Spectro TYPER Ver. 4.0 software (Sequenom, San Diego, CA). Call rates of genotyping were > 95% for the SNP. A total of 120 (5%) samples were randomly selected for the concordance test, and the concordance rates were > 99% for the SNP.

Statistical analysis

Statistical analysis was conducted with SPSS Ver. 17.0 (SPSS, Chicago, IL). Continuous variables were presented as mean and SD or SEM, and compared by Student's $t$test, paired t test or analysis of variance (ANOVA) with post hoc least-significant differences$t$ test. Categorical variables were presented as percentages, and compared by $\chi 2$ test with Bonferroni correction when appropriate. Multivariable Logistic regression was used to analyze the relationship between SNPs and endpoint events in different populations; the degree of association was expressed by odds ratio (OR) and 95% confidence interval (CI); The interaction between gene polymorphism and risk factors was substituted into the equation. Hardy-Weinberg equilibrium for rs3801150 was tested using $\chi 2$ goodness-of-fit test. P values were two-tailed and considered significant when less than 0.05.

Result

Characteristics of the study population

The baseline characteristics of the subjects were shown in Table A1. There were 1993 cases in the MS group and 1079 cases in the control group. The sex and age of the control group and the MS group could be matched. During the 5-year follow-up, 325 subjects were lost to follow-up, 1615 patients in the MS group and 1039 patients in the control group were completed. (Fig. 1).

Genotype analysis was performed on NC_000007.14:g.139985896C>T of the 3072 samples. Finally, there were 1991 cases in the MS group and 1076 cases in the control group completed. The frequency distribution of NC_000007.14:g.139985896C>T genotypes and alleles in control group and MS group was basically consistent with that in HAPMAP-CHB, the frequency distribution of NC_000007.14:g.139985896C>T in both groups did not deviate from Hardy-Weinberg equilibrium. Table A2 presents the genotype and allele frequencies of the NC_000007.14:g.139985896C>T in the MS group and controls, there were no significant difference in the NC_000007.14:g.139985896C>T genotype or the allele distributions between MS and controls ($P > 0.05$). The comparison of baseline clinical characteristics between different genotypes in the control group and the MS group showed that there was no significant difference in clinical indicators between different genotypes in the MS group (all $P > 0.05$). But significant difference was observed in the control group, compared with CC genotype carriers, the CT genotype carriers had significantly higher low-density lipoprotein cholesterol (LDL-C) (P <0.05). (Table 1).

Relevance of SNP and Components of MS

The components of MS include TG, blood pressure, fasting plasma glucose (FPG), abdominal obesity, HDL-C, the relationship between the NC_000007.14:g.139985896C>T genotypes and the components were analyzed (Table 2). The results showed that there was no significant difference between the groups according to TG, blood pressure, FPG, and abdominal obesity. (all $P > 0.05$). It suggested that there was no association between genotypes and these components. But in group of HDL-C, significant differences were observed in the distributions of the CC genotype and the C allele of NC_000007.14:g.139985896C>T between normal HDL-C group and abnormal HDL-C group ($P = 0.023$ & $P = 0.018$, respectively). The HDL-C abnormality of the C allele is significantly higher than the T allele carriers ($P < 0.05$).

Relevance of SNP andendpoints of MS

The relationship between the NC_000007.14:g.139985896C>T genotypes and the endpoints were analyzed of the whole population, MS group and controls. The results showed that there was no significant difference between all-cause death, coronary heart disease, myocardial infarction, new-onset diabetes, new-onset hypertension and new-onset MS in the whole population and MS group ($P > 0.05$), but significant differences were observed in ischemic stroke and the genotypes of NC_000007.14:g.139985896C>T (all $P < 0.05$), especially in the MS group (Table 3). The incidence of ischemic stroke was significantly higher in G allele carriers than in C allele carriers (all $P < 0.05$). In the control group, no endpoint was associated with this locus (all $P > 0.05$).

Risk factors of the onset of ischemic stroke

The Logistic regression analysis was performed for screening the risk factors of the onset of ischemic stroke (Table 4). The results showed that, in the whole population, C allele and gender was the protective factors of the onset of ischemic stroke, the risk of ischemic stroke was decreased when the individual was women or with C allele. SBP was the risk factor for the onset of ischemic stroke, the risk of ischemic stroke was increased when the SBP was higher; in the MS population, C allele and gender was the protective factors of the onset of ischemic stroke, the risk of ischemic stroke was decreased when the individual was women or with C allele. The interaction effects between C allele and the components of MS on the onset of ischemic stroke showed that, in the whole population, there were negative interactions between C allele and WC, SBP, DBP, TG, HDL-C and FPG; in the MS group, there were also negative interactions between C allele and WC, SBP, DBP, TG, HDL-C and FPG. In the case of same WC, SBP, DBP, TG, HDL-C and FPG, the onset of ischemic stroke of C allele carrier were reduced (Table 5).

Discussion

The current researches on candidate genes for MS are mainly focused on genes related to lipid metabolism disorders, hypertension and so on, when these genes related to different components of MS have polymorphisms or mutations, it may touch some risk factors or protective factors of MS, thereby affecting the development of MS. In the early years, researches on *TBXAS1* gene mainly focused on inflammation, breast cancer and other diseases. In recent years, researches on this gene and diseases such as myocardial infarction, stroke, preeclampsia, has become a hot spot. Ulrich et al. [27] found that mutations in the *TBXAS1* gene could alter protein function, which were related to inflammation and angiogenesis. Abraham et al.[28] found that the NC_000007.14:g.139959792T>A mutation of the *TBXAS1*gene could moderately increase the risk of breast cancer. Lemaitre et al. [24] found NC_000007.14:g.139959792T>A was also related to ischemic stroke. Park et al. [29]found that the +16184G> T polymorphism of TBXAS1 gene and TBXAS1-ht3 polymorphism frequency in cerebral infarction, especially in patients with small arterial occlusive cerebral infarction, increased significantly. In our study, there was polymorphism in the NC_000007.14:g.139985896C>T in the Han population in Shandong province of China. Comparison of the genotype and allele frequency distribution of the population suggested that there was no association between MS and the different genotypes of NC_000007.14:g.139985896C>T. However, MS is a syndrome of abdominal obesity, hyperglycemia, dyslipidemia and hypertension. This study observed and recorded the main clinical indicators related to the components of the MS. The results showed that in the MS population, there was no correlation between NC_000007.14:g.139985896C>T polymorphism and clinical indicators. This study further studied the genotype and allele frequencies and the different components of MS. The results showed that this gene polymorphism had no relationship with TG, blood pressure, FPG, abdominal obesity, and HDL-C, suggesting that NC_000007.14:g.139985896C>T polymorphism has no correlation with the incidence of MS. But although there was no difference in HDL-C levels among different genotype carriers, there were differences in HDL-C levels between the C and T alleles, suggesting that the C allele may have a regulatory effect on HDL-C. Therefore, is NC_000007.14:g.139985896C>T related to the prognosis of MS? To this end, we followed the population for 5 years to observe the relationship between the gene polymorphism and the prognosis of MS. This study found that NC_000007.14:g.139985896C>T polymorphism in the general population and MS population is associated with the incidence of ischemic stroke, which was similar to Lemaitre's results that

NC_000007.14:g.139985896C>T polymorphism is associated with ischemic stroke [24]. Recent studies also found that,*TBXAS1* gene polymorphisms had significant association with large-artery atherosclerosis stroke susceptibility and the level of*TBXAS1* expression [30]. A study of Chinese people found that the TT genotype of *TBXAS1* and T allele of NC_000007.14:g.139845571T>G increase susceptibility to ischemic stroke [31]. However, the population of our study was more special, which were MS patients. At the same time, through screening of NC_000007.14:g.139985896C>T polymorphism and risk factors for ischemic stroke, it was found that the C allele was an independent protective factor for the incidence of ischemic stroke in the whole and MS population, individuals who carry the C allele have a reduced risk of stroke. Carriers of T allele are more likely to have ischemic stroke, but that was not an independent risk factor for ischemic stroke in this study, which may be influenced by other factors and need further study. Therefore, people carrying the C allele are less likely to suffer from ischemic stroke, which is more obvious when combined with MS. Patients with T allele should be better treated for MS to prevent the occurrence and development of ischemic stroke. The components of MS, such as hypertension, glucose and lipid metabolism, are genetically predisposed [10, 11]. The heterogeneity of clinical phenotypes suggests that there may be genetic heterogeneity in MS, but no candidate genes have been reported could directly explain the onset and prognosis of MS. These genes mainly affect the occurrence and development of MS through the intermediate phenotype of MS development. Therefore, in this study, interaction analysis was performed of the NC_000007.14:g.139985896C>T polymorphism and the clinical characteristics of the subjects. Obesity is a risk factor of stroke [32], the results of this study showed that there were negative interactions between C allele and WC in the whole and MS population, suggesting that the C allele is a protective factor of ischemic stroke. With constant WC, the risk of ischemic stroke was reduced in carriers of the C allele. Hypertension is also a risk factor for stroke [32]. In the present study, with the genotype of the whole and MS population unchanged, those with high SBP or DBP have a high risk of stroke, which is consistent with previous researches. Abnormal glucose and lipid metabolism are the main clinical feature of MS. In this study, the C allele had negative interactions with TG, HDL-C, and FPG. With the same TG, HDL-C, and FPG, the C allele carriers had a reduced risk of ischemic stroke. It is worth noting that the interaction of genes and clinical characteristics refers to combined effect, and cannot simply add the effects of various factors together. At the same time, a distinction should be made between biologically relevant and statistically relevant differences. Therefore, the statistical interactions found by association analysis need further study of its molecular mechanism to clarify its biological significance.

Some limitations in this study need to be considered. First, the hypothetical molecular mechanism how NC_000007.14:g.139985896C>T polymorphism affected the onset of ischemic stroke was not addressed in the present study. Second, further validation for the predictive value of this SNP as risk factor of MS components and pharmacogenetic indicator in independent cohorts.

Conclusions

This study has indicated that NC_000007.14:g.139985896C>T polymorphism was not associated with the pathogenesis or components of MS. However, it was related to the incidence of ischemic stroke in the whole and MS population, individuals who carry the C allele of NC_000007.14:g.139985896C>T have a reduced risk of stroke. These results suggest that this locus could be used as a therapeutic target for MS and providing a basis for genotype-guided individualized medication.

Disclosure statement

The authors have no conflicts of interest.

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Web Resources

PubMed:https://pubmed.ncbi.nlm.nih.gov/

UCSC:https://genome.ucsc.edu/

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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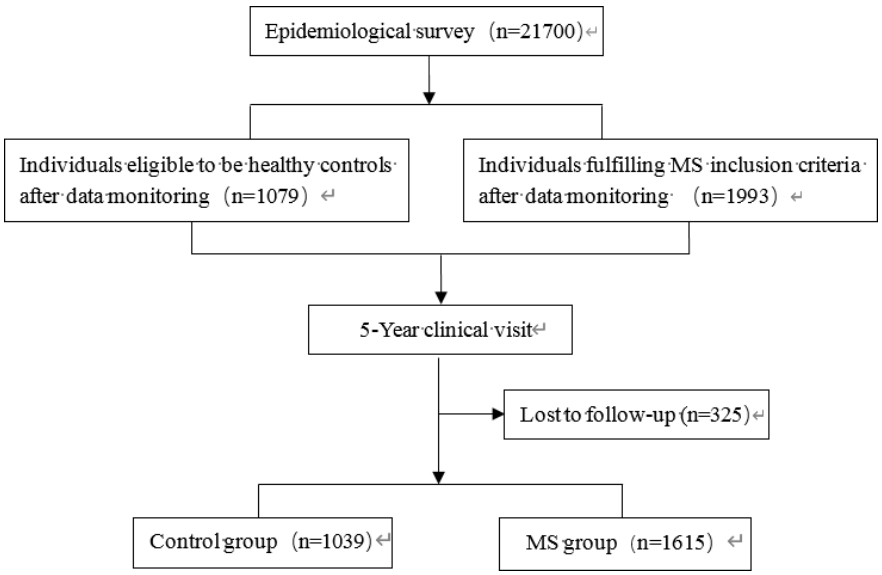
Figure legends

Figure 1. Disposition of Subjects



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