ORIGINAL ARTICLE

Association of GSTT1 and GSTM1 polymorphisms with blood pressure: A Bayesian modeling of continuous data

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Background: Systolic blood pressure (SBP) and diastolic blood pressure (DBP) are a multi-factorial traits and significantly heritable. Glutathione S-transferase (GST) enzyme is involved in detoxification of reactive oxygen species. The present study aimed at finding out the association between GSTM1 and GSTT1 polymorphisms and mean arterial pressure (MAP) in Iranian population. MAP, as the important indicator of blood pressure, is calculated by weighted averaging of SBP and DBP. **Materials and Methods:** we randomly selected 72 healthy individuals from Isfahan Cohort Study (ICS). Polymerase chain reaction (PCR) was done to detect polymorphism of the GSTM1 and GSTT1 genes. The Bayesian Structured Regression model was used, adjusted for sex, age, body mass index (BMI), and smoking status. **Results:** The results showed that both the GSTT1and GSTM1genotypes deletion had a significant effect on MAP increasing in our samples based on 95% Bayesian credible intervals. **Conclusion:** This study demonstrated that GSTT1 and GSTM1 gene increase the arterial pressure; hence, it can predict the susceptibility to cardiovascular disease.

Key words: Bayesian modeling, blood pressure, GSTM1, GSTT1, oxidative stress, polymorphism

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INTRODUCTION

Essential hypertension, a progressively serious worldwide public-health challenge, represents one of the most important risk factors for myocardial infarction, stroke, endstage renal disease, and peripheral vascular disease. Environmental and genetic risk factors and their interaction are known to predispose to hypertension. It has been predicted that ~20-60% of the inter-individual variation of blood pressure (BP) was genetically controlled.[1] Hence, several studies have designed to find the potential hypertension-susceptibility genes for a better understanding of disease etiology.

At molecular level the underlying mechanisms of hypertension are complex, involving many interacting systems such as the renin-angiotensin system, reactive oxygen species (ROS), vascular inflammation and remodeling.[2] The imbalance between prooxidants and antioxidants results in oxidative stress, which is the pathogenic outcome of oxidant overproduction that overwhelms the cellular antioxidant capacity.

Strong experimental evidence indicates that oxidative stress plays an important pathophysiological role in the development of hypertension.[3] The increased production of ROS, reduced nitric oxide synthesis, and decreased bioavailability of antioxidants have been demonstrated in both experimental and human hypertension.[4,5]

Among the physiological antioxidants, glutathione (GSH) is the most abundant intracellular thiolprotein. Reduced GSH scavenges ROS along with regeneration of other antioxidants from their oxidized forms. Through these processes, GSH is converted to its oxidized form (GSSG), which must be reduced by NADPH-glutathione reductase. The cell's ability to conserve GSH levels is very important for its integrity and cellular function.[6] Moreover, GSH is also an essential cofactor for different enzymes like glutathione S-transferases (GSTs).[7] GSH is involved in detoxifying multiple compounds through conjugation to GST. GSTs supergene family are responsible for the regulation of inflammation and oxidative stress.

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The human cytosolic *GST* super family consists of at least 16 genes sub categorized into eight classes designated Alpha, Kappa, Mu, Pi, Sigma, Theta, Zeta, and Omega.[8] Differences in susceptibility to various forms of disease and outcome have associated with polymorphisms of GSTM1, GSTT1, and GSTP1.[9] Three different alleles are found at the locus of GSTM1 (1P13.3) including gene deletion (GSTM1-0) and two functional mutations (GSTM1a and GSTM1b).[10] The human GSTT1 gene has been mapped to chromosome 22q11.2. GSTT1-0 allele represents deletions of the gene.[11] Individuals with homozygous deletions at the GSTM1 and GSTT1 loci (GSTM1-null and GSTT1-null) have no functional enzymatic activity.[12,13] It has been shown that null genotypes of GSTs are important factors in cell sensitivity to Oxidative Stress (OS) and susceptibility to cardiovascular and metabolic disorders.[14,15]

The association between the variations in the GST activity *in vivo* and the susceptibility to cancer, cardiovascular, and other diseases has been studied primarily by the use of homozygous GSTM1-null and GSTT1-null genotypes, as indicators of a systemic lack of expression of the corresponding proteins.[16] The present study was designed to investigate the association of GSTT1 and GSTM1 gene polymorphism with BP in the Iranian population.

MATERIALS AND METHODS

Subjects

This study was performed after approval of the ethical committee of Isfahan University of Medical sciences. A retrospective cohort study of the relationship between GST gene polymorphism and BP for the period between 2002 and 2007 was performed.

Within Isfahan Cohort Study (ICS), which is a study including randomly selected participants from the community of three counties in central part of Iran in the first stage of Isfahan Healthy Heart Program (IHHP), we randomly selected 72 healthy individuals with more complete information. Our subjects were healthy according to their medical history and new measurements at the time of this investigation. We excluded individuals who had: intrinsic renal disease, diabetes, history of cancer, asthma, and a self-reported history of hypertension that was corroborated by the family physician, or had coexisting illness.

The BP was measured in the right upper arm with a standard sphygmomanometer in a sitting position. Systolic blood pressure (SBP) was taken at the return of arterial sounds and diastolic blood pressure (DBP) at the disappearance of sound.[17]

Subject's blood samples were obtained and sequenced; we will describe this later. After 7 years, BP of each individual was again measured. As indices at 2002 related to gender, age, body mass index [BMI: (weight kg)/(height m)^2], SBP, DBP, total cholesterol, high-density lipoprotein (HDL) cholesterol, fasting blood glucose, cigarette smoking habit (no smoking, quit, smoking) were investigated.

Genotyping

DNA was extracted from peripheral blood using blood mini kit (PrimePrep Genomic DNA Isolation Kit, Genet Bio Inc.). For analysis of GSTT1 and GSTM1 genotypes of the subjects, polymerase chain reaction (PCR) amplification was performed using the following primers: GSTT1 forward primer 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′; and reverse primer 5′-TCA CCG GAT CAT GGC CAG CA-3′; GSTM1 forward primer 5′ AGA CAG AAG AGG AGA AGA TTC 3′; and reverse primer 5′ TCC AAG TAC TTT GGC TTC AGT 3′. The PCR reaction was done as previously described.[18]

Statistical analysis

We develop a Bayesian Structured Regression model for investigating the effect of some genotypes deletion on hypertension incidence. Bayesian inference scheme uses the posterior distribution, that is, the conditional distribution of the model parameters given the observed data. In this study, we use a fully Bayesian inference based on analysis of posterior distribution of the model parameters. Monte Carlo Markov Chain (MCMC) was used for estimation and this was implemented in the freely available software WinBUGS (BUGS project, http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/). Gibbs sampler was employed for MCMC simulations, drawing successively from the full conditionals for the variance components and unknown parameters. We used the total number of 100,000 MCMC iterations with 20,000 burn in samples. Since, in general, these random numbers are correlated, only every 10th sampled parameter of the Markov chain were stored. Convergence was assessed by visual inspection of the means in time-series plots but also more formally using the Gelman — Rubin R-hat diagnostics.[19] Our goal was showing the association between GSTM1 and GSTT1 genotypes deletion status and mean arterial pressure (MAP) changes as an indicator for hypertension adjusted by the effect of sex, age, BMI, and smoking status of patients. All prior distributions were chosen to be as uninformative as possible.

RESULTS

Descriptive statistics as percentages and mean ± SD showed that 49% of total patients were male and 51% were female. The positive rate for smoking was 20%. The BMI and age variables as continuous variables were 29.5 ± 13.6 and 57.5 ± 9.9, respectively. Table 1 contains the posterior means, posterior standard deviations, and 95% credible intervals for the parameters of interest. The results showed

Table 1: Posterior results for the final Bayesian model for showing effective genotypes deletion on mean arterial pressure (MAP) adjusted by BMI, age, sex, and smoking status

Parameter	Mean	SD	95% Credible intervals
GSTT1	2.17	0.92	(0.37-3.97)
GSTM1	1.56	0.78	(0.02-3.10)

that both the GSTM1 and GSTT1 genotypes deletion had a significant effect on the MAP increasing in our samples based on 95% credible intervals [Table 1].

DISCUSSION

This study has investigated *GSTM1* and *GSTT1* gene polymorphisms in Iranian population with the incidence of hypertension and demonstrated that both the *GSTM1* and *GSTT1* genotypes deletion had a significant effect on the MAP increasing in our sample based on 95% credible intervals.

It is well established that high BP is a risk factor for coronary heart disease. Several studies show that between 30% and 40% of BP variation in a population is thought to have a genetic basis.[20]

The importance of oxidative stress in hypertension has recently reserved more attention. Many studies have generally supported the idea that hypertension is associated with increased vascular oxidative stress; however, human studies were in conflict.[21,22] Oxidative stress may produce and maintain hypertension via several mechanisms like, quenching of the vasodilator nitric oxide by ROS such as superoxide; generation of vasoconstrictor lipid peroxidation products; and structural and functional alterations within the vasculature.[23]

The GSTs are involved in the detoxification of many toxic compounds of different chemical structures in cigarette smoke, including epoxybutane, ethylene oxide, monohalomethane, and reactive metabolites of polycyclic aromatic hydrocarbons, such as benzo[a] pyrene.[24,25] They act as antioxidant through inactivation of endogenous unsaturated aldehydes, quinines, epoxides, and hydroperoxide formed as secondary metabolites during the oxidative stress, thus playing a key role in protecting cell types of various origins, including vascular smooth muscle cells and endothelial cells against oxidant damage.[8,26] In humans, there is a wide variation in GST activity due to genetic polymorphisms, which can result in oxidative stress potentiation and influence the individual's susceptibility to diseases, including hypertension.[27,28]

In our study, by using Bayesian Structured Regression model and adjusted by the effect of sex, age, BM,I and smoking status, in subject with deletion of two classes of GSTs, GSTT1 and GSTM1, the average of arterial pressure had increased from 2001 to 2007. MAP is a weighted average of SBP and DBP and is strong prognostic predictors of adverse cardiovascular events.[29] Several genetic loci have been reported to be associated with SBP and DBP.

Several studies have shown the association of this genotype with hypertension. Oniki *et al*. observed that the risk of hypertension was significantly increased in the GSTA1*B allele carriers that also had the GSTM1-null genotype or both the GSTM1 and GSTT1-null genotypes.[30] Capoluongo *et al*. reported that GSTM1-null variants were significantly associated with hypertension in elderly subjects.[31] In addition, Saadat and Dadbine-Pour showed an influence of GSTM1 polymorphism on SBP in normotensive individuals and modulation of BP in individuals chronically exposed to natural sour gas containing sulfur compounds).[32,33] Moreover, the results of Tew *et al*. described a higher frequency of GSTM1 and GSTT1-null genotypes (especially GSTT1-nulls) among systemic sclerosis patients with hypertension and pulmonary involvement.[34] Bessa *et al*. reported that the frequency of GSTM1 and GSTT1positive was significantly lower in essential hypertensive patients than in normotensive subjects.[35]

These observations proposed that a genetic background (GST gene polymorphisms) may contribute to the development of hypertension. The most probable explanation is based on the antioxidant activity of GST enzyme. It has been reported that reduction of GSTM1 expression in the stroke-prone spontaneously hypertension rat contributes to increased oxidative stress.[36] Based on experimental evidence and clinical studies that oxidative stress plays a key role in vascular damage, there has been a great interest in developing strategies that target ROS in the treatment of hypertension. Therefore, the oxidative stress might be the necessary link between GSTs activity and hypertension. It is worth noting that deleted polymorphisms in the GST genes may also influence the susceptibility to coronary artery disease by modulating the detoxification of genotoxicatherogens.[37]

In addition, our result is in conflict with some previous studies.[31,38,39] The heterogeneity in the outcomes for the GST genes and BP could be due to extreme gene — environment interactions that characterize the hypertensive phenotypes but could also be related to the difference in the selection of cases and controls. Further studies to understand the role of genetic susceptibility to oxidative stress in the development of hypertension are merited.

AUTHORS' CONTRIBUTION

L.R carried out the genetic experiments and prepared the manuscript. M.A conducting the statistical analysis. N.S and

S.H.J contributed to the study design, conducting the study, and approving the manuscript. M.M interpreted the results and editing the manuscript. All authors read and approved the final version of the manuscript.

CONCLUSIONS

In conclusion, the genetic polymorphism of GSTT1 and GSTM1 by adjusting effect of sex, age, BMI, and smoking status was significantly associated with the average arterial pressure increasing suggesting that the GSTM1 and GSTT1 genes are the candidate genes that alter BP and subsequently, the susceptibility to atherosclerosis with regard to sex, BMI, and cigarette smoking.

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