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Relationship of Serotonin Transporter Gene Polymorphism With Temperament in Persian Medicine in Fars Province, Iran

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ABSTRACT

Background: Despite its effectiveness, there are still many concerns and questions about the principles and therapeutic methods of Traditional Medicine (TM). In other words, to accept TM as a reliable healthcare system, modern scientific research should approve the principles of this system. Temperament is one of the major determinants of Persian Medicine (PM), which is practically utilized to diagnose and cure diseases. Considering the association of depression with the coldness temperament and the serotonin-Tansporter-Linked Promoter Region (5-HTTLPR) polymorphism, we proposed that the serotonin transporter gene could be one of the candidate genes contributing to the specification of temperament. To test this hypothesis, we examined the association of 5-HTTLPR polymorphism with the hot/cold status of temperament in healthy individuals.

Materials and Methods: The study included 351 healthy men (aged 20-40 years) referred to the organization of blood transfusion in Shiraz City, southern Iran. The participants' temperament (warm, temperate, and cold) was determined using a standard self-reported scale. Then, we performed Polymerase Chain Reaction (PCR) to determine their 5-HTTLPR polymorphism genotype. Multinomial logistic regression was used to evaluate a 95% CI and odds ratios for the association of temperament with the 5-HTTLPR genotypes. Statistical analysis was performed using the SPSS at a significance level of less than 0.05.

Results: Regarding the warm temperament, no association with the 5-HTTLPR genotypes was observed. However, regarding cold temperament, although our data showed no association with the SS, the LS genotype showed some association. With reference to LL, the LS genotype decreased the possibility of coldness rather than temperateness for the temperament (OR=0.471, P=0.040).

Conclusion: Our data revealed the association of temperament with the 5-HTTLPR polymorphism, suggesting that the serotonergic system may influence temperament. Further studies are required to explore the relation of genetic factors with temperament.

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Introduction

In many parts of the world, Traditional Medicine (TM) is one of the important systems of healthcare service [1]. One of the major determinants of Persian Medicine (PM) is temperament ("mizaj" in native language), which is practically utilized in the diagnosis and cure of diseases. As stated in the PM, temperament is regarded as a homogenous quality from a combination of fire, air, earth, and water with the qualities of hot and dry, hot and wet, cold and dry, and cold and wet in the human. Thus, concerning the degree of warmness/coldness and wetness/dryness, many temperament types could be considered. Nevertheless, for easiness, PM assorts the types of temperament into 9 main groups: four simple types (warm, cold, moist, and dry), four compound types (warm-moist, warm-dry, cold-moist, and cold-dry), and one temperate type (equilibrium) [2, 3]. The wisdom of PM is essentially derived from old science texts and useful experiences; thus, despite its effectiveness, a lot of questions and concerns about the principles and therapeutic methods of PM have remained unanswered. In other words, to accept TM as a reliable healthcare system, modern scientific research should support its proficiency.

It is believed that morphological, physiological, and psychological characteristics of human individuals are associated with their "mizaj" [3]. Several recent research studies indicated the connection of temperament with various biological aspects, such as fat and skeletal muscle mass [4, 5], mood state [6], enzymatic and hormone variation [7, 8], serum bilirubin [9], basal metabolic rate, systolic and diastolic blood pressure [5], genetic factors [10, 11], asymmetry of the digit ratio [12], and so on.

Serotonin is a neurotransmitter involved in various central nervous system functions, including behavior and mood. The serotonin transporter is located on the plasma membrane of presynaptic cells. It eliminates serotonin from the synapses and significantly controls synaptic serotonin signaling and concentration [13, 14]. Disruption in serotonin transmission is linked to depression [15]. The gene encoding human serotonin transporter (SLC6A4) is located on chromosome band 17q11.2 (OMIM: 182138). There is a polymorphism of 20-23-bp repeat elements in the promoter region of the serotonin-transporter-linked promoter region (5-HTTLPR), creating typical short (S, 14 repeats) and long (L, 16 repeats) alleles [14]. However, other lengths have also been reported [16]. The S and L alleles resulted in lower and higher gene expression, respectively [14]. This polymorphism is associated with suicidal behaviors and depression [17]. In the view of PM, depression, and hopelessness are related to the coldness of "mizaj" [3, 18].

We recently examined the association of depression and hopelessness with the temperament in the women and found higher depression and hopelessness scores in the group with cold temperament compared to that with the warm one [19]. Considering the association of depression with the coldness of temperament and the 5-HTTLPR polymorphism, we proposed that the serotonin transporter gene could be one of the candidate genes contributing to the specification of temperament. To test this hypothesis, we examined the association of 5-HTTLPR polymorphism with the hot/cold status of temperament in healthy individuals. It should be mentioned that since the body properties are more affected by the hot/cold state than the wet/dry one [3], we focused on the hot/cold status of the temperament in this study.

Materials and Methods

Study population

The study included 351 healthy male blood donors referred to the organization of blood transfusion in Shiraz City, southern Iran. They were aged 20-40 years. To detect the participants' hot/cold status of temperament, we used a standard self-report questionnaire to categorize the temperament of the respondents into three groups of warm, temperate, and cold [20]. Informed consent was obtained from each volunteer, and the study was approved by the Ethics Committee of Shiraz University (ECBDE-SU-9-6177616).

Genotyping

Genomic DNA was obtained from blood samples. Briefly, the whole blood was treated with 170 mM NH4Cl in a 1.5-mL tube, and then the cells were washed with 10 mM EDTA-NaCl. Next, the cells were treated with 50 mM NaOH, and the tube was placed in boiling water for 5 min. Then, 1 mM Tris-HCl was added, and after centrifugation, the supernatant was used as a solution containing DNA. To genotype the 5-HTTLPR polymorphism, Polymerase Chain Reaction (PCR) was done using the following primers: F, 5'-ATGTCCCTACTGCAGCCTCC and R, 5'-AGTCCGCGCGGGATTC. The PCR steps were 15 min of initial denaturation at 94°C; 40 cycles, including 1 min of denaturation at 94°C, 50 s of annealing at 65°C, and 40 s of extension at 72°C; and 10 min of final extension at 72°C. The products of PCR were 440 and 396 bp fragments, detected by 1.7% agarose gel stained with ethidium bromide (Figure 1).

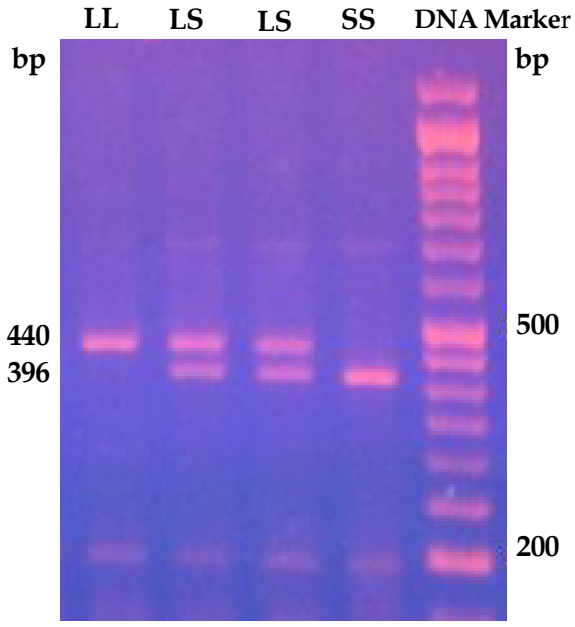

Figure 1. Agarose gel displaying the resultant PCR products

An individual with LL genotype holds 440-bp fragment, LS genotype holds 440- and 396-bp fragments, and SS genotype holds 396-bp fragment.

Statistical analysis

The Court lab-HW calculator software was applied to check the Hardy-Weinberg equilibrium. The mean age difference across the study groups was accessed using ANOVA. Multinomial logistic regression was used to evaluate 95% CI and odds ratios (ORs) for the association of temperament with the 5-HTTLPR genotypes. Statistical analysis was performed using the SPSS version 26 at a $P<0.05$.

Results

After genotyping, 82, 164, and 105 individuals held LL, LS, and SS genotypes, respectively. The frequencies of total allelic of the 5-HTTLPR polymorphism were 47% of L and 53% of S alleles, and the sample followed the Hardy-Weinberg equilibrium ($\chi^2=1.3$, df=1, $P=0.249$).

The study included 146, 74, and 131 individuals with warm, cold, and temperate temperament with the Mean±SD ages of 31.1±4.9, 31.7±5.1, and 32.3±5.4 years, respectively. No significant difference was detected between the temperament groups in terms of the mean age ($F=1.797$; df=2, 348; $P>0.05$). In multinomial regression, the temperate group and the LL genotype were assumed as the reference categories for dependent and independent variables, respectively (Table 1).

Table 1. Association of hot/cold status of temperament with the Serotonin-Transporter-Linked Promoter Region (5-HTTLPR) polymorphisms

5-HTTLPR Genotype	Temperate	Warm	Cold	B	OR	97% CI	P
LL	25	35			1	-	-
LS	70	65		-0.411	0.663	0.359-1.226	0.190
SS	36	46		-0.091	0.913	0.465-1.790	0.790
LL	25		22		1	-	-
LS	70		29	-0.753	0.471	0.230-0.965	0.040
SS	36		23	-0.320	0.726	0.334-1.577	0.419

B: Coefficient; OR: Odds Ratio; CI: Confidence Interval

In terms of a warm temperament, no association with the 5-HTTLPR genotypes was observed. However, in terms of cold temperament, our data showed no association with the SS but with the LS genotype. With reference to the LL, the LS genotype decreased the possibility of coldness rather than temperateness for the temperament.

Discussion

Because of its effectiveness and convenience, traditional medicine is the main and sometimes the only healthcare system for many people around the world [1]. Based on PM, temperament functions as an essential map in lifestyle and health directives and medical decisions on diagnosing and treating illnesses [3]. Any alteration in the temperament may deviate a human body from its health condition. In other words, keeping temperament in a balanced state is essential for perpetuating health and avoiding diseases [2]. For instance, it is believed that dystemperament in the uterus leads to its inability to hold the embryo [21], individuals with cold dystemperament of the intestine have weak digestion [22], and people with wet dystemperament of the liver suffer from fatigue and frailty [23]. Because of the special contribution of temperament in health maintenance, temperament adjustment is one of the regular remedy methods for sicknesses in the PM health care system [2].

Recent studies imply the correlation of "mizaj" with individual genetic factors. It has been reported that the genes correlated to the cold syndrome are associated with energy metabolism [24]. Exploring the profiles of the CD4+ T cells gene expression in patients with a hot or cold pattern of rheumatoid arthritis showed that some genes in the patients with hot patterns expressed differently from those in the patients with cold patterns. On the other hand, an increased expression of the genes involved in the metabolism of fatty acid, T cell proliferation, and pathways of small G protein signaling was detected in the patients holding hot patterns of the diseases [11]. In a study of proteins extracted from the mitochondrial of peripheral blood mononuclear cells, some proteins are related specifically to each of hot-wet or cold-dry temperaments group, and some of them overexpressed differentially in specified group [25]. It has been reported that genes linked to the hot ZHENG-associated diseases are chiefly present in the pathway of cytokine–cytokine receptor interaction [26]. In this study, we examined the association of 5-HTTLPR polymorphism with the hot/cold status of the temperament in apparently healthy men. Serotonin involves controlling many physiological and behavioral activities, and the serotonin transporter could affect the function of this signaling molecule by altering serotonin synaptic concentration [15, 27]. Our data showed no association between warm temperament and 5-HTTLPR genotypes.

With regard to the cold temperament, our results showed no association between cold temperament and the SS genotype, but with the LS one. With reference to the LL, LS decreased the possibility of coldness rather than temperateness for a person's temperament. This result indicates that "mizaj" may be affected by the serotonergic system. It is noteworthy that we previously examined the relationship of temperament with the gene polymorphisms in the *GSTM1/T1* and *SOD1*, encoding antioxidant enzymes, and revealed a higher frequency of *GSTT1*-null genotype in individuals with warm temperament compared to whom which were temperate for the warmness [10, 28, 29]. Our studies, together with other genetic studies related to temperament, suggest that the temperament could be, at least partially, under the control of the genes. Indeed, identifying the genes involved in the determination of this trait could improve the understanding of biological mechanisms of the temperament-based treatment in PM, which may consequently lead to profit more from this medical system.

5. Conclusions

Our data revealed that with reference to the LL, the LS genotype decreased the possibility of coldness rather than temperateness for a person's temperament. This finding suggests that the serotonergic system may influence temperament. To our knowledge, this study is the first of its kind, so further research with larger samples is required to identify the genetic factors associated with the temperament.

Ethical Considerations

Compliance with ethical guidelines

Informed consent was obtained from each volunteer, and the study was approved by the Ethics Committee of Shiraz University (ECBDE-SU-9-6177616).

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Authors' contribution's

Methodology, Data collection, Data analysis and Writing – original draft: Shabnam Aliabadi; Writing – review & editing: Zahra Zendehboodi.

Conflict of interest

The authors declared no conflict of interest.

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