

No Association between 5-HTTLPR and Harm Avoidance in Korean College Students

There have been numerous studies on the association between 5-HTTLPR (polymorphisms in the promoter region of the serotonin transporter gene) and anxiety-related personality traits, with conflicting results. In this study, we administered Korean version of the Temperament and Character Inventory (K-TCI) to a sample of 158 Korean college students and genotyped for the 5-HTTLPR in order to compare the TCI dimensional scores including harm avoidance according to the 5-HTTLPR genotype and sex. We could not find the association between 5-HTTLPR and harm avoidance and other TCI measures. Considering known allele frequencies differences of 5-HTTLPR among different ethnic groups, further cross-cultural studies with a larger sample would be needed.

Key Words : Anxiety-related personality trait; 5-HTTLPR; Temperament and Character Inventory; Harm Avoidance; Korea; SLC6A4 protein, human; Polymorphism, Genetic

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INTRODUCTION

Serotonin transporter gene (SLC6A4, solute carrier family 6, member 4) encodes an integral plasma membrane protein that plays a major role in the regulation of serotonin neurotransmission (1). It removes serotonin from the synaptic cleft into the pre-synaptic terminals. It has been widely recognized as the target sites of selective serotonin reuptake inhibitor (SSRIs), which have been recognized as both anti-depressant and anti-anxiety drugs. Two common polymorphisms in the promoter region of the serotonin transporter gene, i.e. 5-HTTLPR (polymorphisms in the promoter region of the serotonin transporter gene) have been reported, together with their functional properties. 5-HTTLPR, containing long ("l") and short ("s") alleles confirmed by conventional polymerase chain reaction and electrophoresis, were involved in the transcriptional regulation of the serotonin transporter gene (2, 3). The short allele has been suggested to decrease transcriptional activities of serotonin transporter gene, although it is uncertain whether they act in a dominant fashion (4) or a recessive fashion (5-7).

Studies have shown that reliably measured personality dimensions are substantially influenced by genetic factors (8). Among them, anxiety-related traits such as harm avoidance or neuroticism appears to be one of the most fundamental,

enduring, and continuously distributed personality dimensions, which are associated with 5-HTTLPR (2, 9, 10). These associations, however, remain controversial, because numerous other studies yielding negative results (11-15). These inconsistencies could be accounted for by various factors including different measures applied, confounding effects of age and gender, and inadequate sample size. Perhaps more importantly, ethnic differences in allele frequencies and population substructure may underlie those inconsistent results.

In the present study, we examined whether association between 5-HTTLPR and harm avoidance of Korean version of the Temperament and Character Inventory (K-TCI) (16) should be replicated in Korean population. Koreans have been considered more ethnically homogenous (17). We also examined other personality traits in K-TCI since other personality dimensions of novelty seeking, reward dependence, and persistence are reported to be independently heritable (18). We confined our sample to one college students, which was presumed to be relatively homogenous population regarding age and sociocultural backgrounds.

MATERIALS AND METHODS

A total of 158 unrelated healthy college students volun-

Table 1. TCI Scores grouped by genotype

Genotype (No./%)	TCI factor scores			
	Harm avoidance	Novelty seeking	Reward dependence	Persistence
"ss" (95/60.1)	17.14±7.11	20.36±6.15	15.40±3.48	4.81±2.13
"ls" (54/34.2)	16.59±7.71	20.28±6.13	15.72±4.31	4.69±2.36
"ll" (9/5.7)	18.78±4.02	20.67±7.58	15.22±1.99	5.22±2.05
F	0.71	0.93	0.23	0.55
<i>p</i>	0.62	0.46	0.95	0.74
"ss" (95/60.1)	17.14±7.11	20.36±6.15	15.40±3.48	4.81±2.13
"ls+ll" (63/39.9)	16.90±7.31	20.33±6.23	15.65±4.05	4.76±2.31
F	1.03	0.01	0.35	0.07
<i>p</i>	0.38	0.99	0.79	0.97

teered the study. They were from Hong-Ik University, located in Seoul, Korea. There were 69 males and 89 females, average age of 23.8 ± 3.1 (mean \pm S.D.) yr. All subjects were Korean ancestry. All subjects participated in the study after giving informed consent. The study protocol was approved by the Institutional Review Board of Asan Medical Center. A K-TCI (16) was administered to the participants.

A 10 mL venous blood sample was obtained, and genomic DNA was isolated from peripheral blood leukocytes according to standard procedures. DNA fragments were amplified by polymerase chain reaction using 5'-GGCGTTGCCGCTCTGAATTGC (-1,416 -> -1,397) and 5'-GAGGGACTGAGCTGGACAACCCAC (-910 -> -889) primers (3). The PCR reaction mixture contained a total volume of 20 μ L with the following composition: 12 ng genomic DNA, 4 pmoles of the each primers, 2.5 mM dNTPs, 5 mM of deaza dGTP, and 1 U Ampli Taq with the appropriate buffer. After an initial 5 min denaturation step at 95°C, 5 cycles were performed consisting of 40 sec at 95°C, 40 sec at 58°C, and 60 sec at 72°C, followed by an additional 35 cycles of 40 sec at 95°C, 40 sec at 61°C, and 60 sec at 72°C. The reaction was ended by incubation at 72°C for 7 min. PCR products were separated by the long (524 bp) and short (484 bp) variants on 3% agarose gels with ethium bromide.

We compared harm avoidance scores across the different genotypes ("ss", "sl" and "ll") and sex. Then, we compared the scores between "ss" group and "sl" + "ll" group, since "s" allele might act as recessive fashion (5, 19). Additionally, we compared another 3 dimensional scores of novelty seeking, reward dependence, and persistence. We applied two way ANOVA using genotype and sex as independent variables. Statistical Package for the Social Sciences (SPSS, version 10.0, SPSS Inc, Chicago, IL., U.S.A., 2000) was used. Multiple testing corrections were not made.

RESULTS

The genotypes of 5-HTTLPR were distributed according to the Hardy-Weinberg equilibrium ($\chi^2=0.13$, $p=0.94$).

Ninety-five subjects (60.1%) were "ss" genotype, and subjects with "sl" and "ll" were 54 (34.2%) and 9 (5.7%), respectively. Neither allele nor genotype frequencies differed according to sex. There were no significant differences in the scores of harm avoidance ($F=0.38$, $p=0.69$), novelty seeking ($F=0.07$, $p=0.93$), reward dependence ($F=0.16$, $p=0.86$) and persistence ($F=0.24$, $p=0.79$) using genotype and sex as independent variables (Table 1). When dividing the subjects into 2 groups of "ss" (60.1%) and "sl" + "ll" (39.9%), we could not find associations between the two genotype group and personality traits, either (Table 1).

While the 5-HTTLPR genotypes frequencies ($\chi^2=111.04$, $p<0.001$) and the allele frequencies ($\chi^2=110.21$, $p<0.001$) in our sample were significantly different from those of Lesch et al. (2), those frequencies are quite similar to other studies of Korean (20, 21), Japanese (6, 12, 13), and Chinese (22).

DISCUSSION

In the present study, we could not find evidence for an association between 5-HTTLPR and harm avoidance measured by K-TCI in healthy Korean subjects. It is contrary to two recent meta-analyses including more than 23 studies which have shown the association between the polymorphism and trait anxiety, despite its weak effects (14, 15). This relationship was not reported in previous two studies using Korean samples (20, 21). Even though the association between 5-HTTLPR and harm avoidance is not confined to one ethnic group (10), majority of studies in East Asian populations did not show the association (20-23), with two possible exceptional studies in Japan (6, 12).

This raises the possibility that the reported associations between the 5-HTTLPR and harm avoidance do not exist in East Asian population. We found that allele frequencies in our subjects were similar to those in other East Asian subjects (6, 12, 13, 20, 21), but they were significantly different from those in Caucasian subjects (2, 5, 24). It is well known that allele frequencies distribution has large effects on the power of genetic association studies (25). Together with small

effect size of harm avoidance (14, 15), it is certainly possible that genotype distribution of East Asian samples, especially marked low frequencies of long alleles, could reduce the power of the tests, which might lead to negative results.

There have been many reports suggesting dominance of long allele (5, 19). There are also reports that "ss" genotype acts in recessive fashion in East Asian populations (6, 7, 26). In accordance with these, we divided the subjects into "ss" genotype group and "sl" + "ll" genotype group. There were no significant differences between the two genotype groups and 4 dimensional scores, either.

The ethnicity issue could be addressed in the context of cultural factors. It has been suggested that high frequency of short allele in the Japanese population may be responsible for the interpersonal sensitivity and emotional restraints that is generally regarded as characteristic of Japanese culture (6, 23). The ethnic differences of allele frequencies between Asians and Caucasians might partially explain personality tendencies towards greater or less cautiousness and shyness from a genetic perspective. However, in our sample, we could not find the correlation between the high scores of harm avoidance and short alleles of 5-HTTLPR.

In terms of gender differences, we could speculate that genetic factors have different effects on personality differences between sex (27). However, we could not find previously reported gender differences (5, 11) in this study. In another Korean study exclusively including female subjects, they could not find association between harm avoidance and 5-HTTLPR (28).

Though TCI has been known to reflect the genetic components of personality dimensions including harm avoidance, it also includes non-genetic, environmental components (29). Considering that age, sex and culture could affect on personality dimensions (30), our study has some strength in that the study subjects could be relatively homogenous regarding ethnicity, age, cultural and socioeconomic status, because we confined our sample to the students of one university in Seoul, Korea. The average age of the participants were 23.8 ± 3.1 , minimizing previously reported age effect on the TCI dimensional scores (31). We also found no correlations between age and personality traits scores in our sample (result not shown). Though college students do not represent entire population, we could reduce potential confounding effects of age and cultural factors on the personality traits.

Overall, we could not find the association between 5-HTTLPR and harm avoidance and other TCI measures. While the allele frequencies of our sample are similar to those of other studies in East Asia including Korea, they were significantly different from those of Lesch et al. (2), which support further cross-cultural replication studies with a larger sample.

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