Functional polymorphism of the GTP cyclohydrolase 1 gene affects the personality trait of novelty seeking in healthy subjects

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A B S T R A C T
GTP cyclohydrolase 1 (GCH1) is the initial and rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin, which is an essential cofactor for biosynthetic enzymes of dopamine, serotonin, and nitric oxide. In the present study, the association of functional polymorphism of the GCH1 gene (C+243T, rs841) with personality traits was examined in 902 healthy Japanese subjects. Personality traits were assessed by the Temperament and Character Inventory (TCI), and the GCH1 genotype was detected by a PCR–RFLP method. There were no significant main effects of the GCH1 genotype on the seven TCI dimension scores, but significant interaction effects between the GCH1 genotype and gender were found on the scores of novelty seeking. Post-hoc analysis revealed that males with the C/T genotype had higher scores of novelty seeking than those with the C/C genotype or those with the T/T genotype, while in females the scores of novelty seeking were not different among the genotype groups. The present study thus suggests that the C+243T polymorphism of the GCH1 gene affects the personality trait of novelty seeking in males.

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GTP cyclohydrolase 1 (GCH1) catalyzes the conversion of guanosine triphosphate to dihydronopterin triphosphate, and is the initial and rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin (BH4) [8] (Fig. 1). BH4 is an essential cofactor for biosynthetic enzymes of several neurotransmitters, i.e., tyrosine hydroxylase, tryptophan hydroxylase, phenylalanine hydroxylase, and nitric oxide (NO) synthase, which catalyze the production of dopamine, serotonin, tyrosine, and NO, respectively [8] (Fig. 1). It has been reported that inherited GCH1 deficient mice show decreased levels of BH4, dopamine, serotonin, and norepinephrine in brain [11] and decreased levels of NO in plasma [13]. Patients with dopa-responsive dystonia or Segawa disease, which is characterized by GCH1 deficiency caused by rare GCH1 gene mutations, have been reported to show decreased levels of BH4 accompanied by low levels of the dopamine metabolite homovanillic acid and the serotonin metabolite 5-hydroxyindoleacetic acid in cerebrospinal fluid [11]. These patients also exhibit various psychological symptoms such as mental retardation, depression, and obsessive-compulsive disorders [8,10]. These studies suggest that GCH1 activity is involved in human mental functions and behaviors.

Human GCH1 gene is located at 14q22.1–22.2 and contains 6 exons. Zhang et al. [22] reported a C+243T single nucleotide polymorphism (C59038T, rs841) in 3′ untranslated region of the GCH1 gene. They showed that the T allele of this polymorphism caused lower luciferase activity in transfected chromaffin cells than the C allele [22]. Furthermore, the T allele was related to lower urinary levels of NO, higher blood pressure and heart rate, and dysfunction of the baroreceptor reflex in vivo [22]. This allele was also associated with lower plasma NO levels and increased risk of vascular diseases in patients with type 2 diabetes [14]. Meanwhile, a haplotype comprising 3 single nucleotide polymorphisms of the GCH1 gene, which is in strong linkage disequilibrium with the T allele of the C+243T polymorphism [8], was associated with lower GCH1 mRNA levels [2,20], reduced BH4 levels in vascular and leucocytes [2,20], and reduced pain sensitivity [20].

The Temperament and Character Inventory (TCI) is a comprehensive personality scale developed from biological perspectives by Cloninger et al. [4]. The TCI has four temperament dimensions (novelty seeking, harm avoidance, reward dependence, and persistence), and three character dimensions (self-directedness, cooperativeness, and self-transcendence). They hypothesized that novelty seeking, harm avoidance, and reward dependence were related to dopamine, serotonin, and norepinephrine, respectively [4]. It has been suggested that several psychiatric disorders, such as anxiety disorders, mood disorders, eating disorders, and substance dependence, are related to specific dimensions of the TCI.
[3,4,5,9]. Furthermore, prospective studies showed that high harm avoidance predicted future depression in a general population [5,9]. Therefore, it is important to identify the factors involved in the characterization of personality traits to promote understanding of the pathogenesis of these disorders.

Taken these discussions all together, it is possible that the C+243T polymorphism in the GCH1 gene affects personality traits. However, there have been no studies examining the relationship between this polymorphism and personality traits. Therefore, we studied the association of this polymorphism with personality traits in healthy subjects.

Originally, 995 unrelated Japanese volunteers without serious physical diseases were recruited from medical students and the staff at five hospitals. Forty-nine subjects with a current or past history of psychiatric disorders according to the DSM-IV [1] were excluded after interview by well-trained psychiatrists. In 14 of the 995 subjects, the GCH1 genotype was not determined due to failure of PCR amplification. Thirty subjects were excluded due to a missing data. Thus, the data for 902 subjects were used for statistical analyses. Four hundred ninety seven subjects were males, and 405 were females. The mean ± SD of age was 27.1 ± 8.2 years. The study protocol was approved by the Ethics Committee of Yamagata University School of Medicine, and all subjects provided written informed consent to participate.

Assessment of personality traits was performed using the Japanese version of the TCI, which has been verified to have high internal consistency and construct validity [12].

The 361 bp in the GCH1 gene was amplified using a set of primers (forward: 5′- GTT GCT TGC CGA TCG TAC TG -3′ and reverse: 5′- CAG TAT ACT GGG CAC AGT TC -3′) in 20 μl volume containing 100 ng of genomic DNA, 0.5 μM of each primer, 200 μM of each dNTP, 1.5 mM of MgCl2, and 1.25 U of HotStar Taq DNA polymerase (Qiagen, Tokyo, Japan). After an initial denaturation step at 95 °C for 1 min, 40 cycles were performed at 94 °C for 0.5 min, 54 °C for 0.5 min, and 72 °C for 0.5 min. Finally, an elongation step was performed at 72 °C for 10 min. Five μl of the PCR product was digested overnight with 10 U of Tail (Cosmo Bio Co, Tokyo, Japan). The fragments were electrophoresed on a 2% agarose gel with ethidium bromide staining and visualized using ultraviolet light. After digestion by Tail, the PCR product containing the C allele is cleaved into 225- and 136 bp fragments, while that containing the T allele remains intact (361 bp fragment).

The Hardy–Weinberg equilibrium for genotype distribution was tested by the chi-square test. Zhang et al. [22] reported a significant interaction effect between the GCH1 polymorphism and gender on blood pressure. Thus, the effects of the GCH1 polymorphism on the TCI dimension scores were tested by the two-factor analysis of variance with the GCH1 genotype and gender as factors and with age as a covariate. In these analyses of seven TCI dimensions, a p value less than 0.05/7 (two-tailed) was regarded as significant according to Bonferroni correction. When the interaction between the GCH1 polymorphism and gender was significant in some TCI dimension, that dimension score was compared among the GCH1 genotype groups in males and females separately, by the one-factor analysis of variance with age as a covariate followed by the LSD test. A p value less than 0.05 (two-tailed) was regarded as significant. All statistical analyses were performed using SPSS 14.0 for Windows (SPSS Japan Inc, Tokyo, Japan).

The distribution of the GCH1 genotypes was in the Hardy–Weinberg equilibrium (χ2 = 0.185, df = 1, p = 0.667) (Table 1). There were no significant main effects of the GCH1 genotype on the seven TCI dimension scores, while significant main effects of the gender were found on the scores of harm avoidance (p < 0.007) and reward dependence (p < 0.007) (Table 1). The interaction between the GCH1 genotype and gender was significant (p = 0.014) on the scores of novelty seeking (Table 1). In the post-hoc analysis, there were significant differences in the scores of novelty seeking among the GCH1 genotype groups in males (F = 6.432, p = 0.002) but not in females (F = 1.190, p = 0.305). Males with the C/C genotype had higher scores of novelty seeking than those with the C/T genotype (p = 0.002) or those with the T/T genotype (p = 0.004) (Fig. 2).

In the present study, the T allele of the C+243T polymorphism in the GCH1 gene was related to lower scores of novelty seeking in males. It is described that high scores of novelty seeking are exploratory, curious, impulsive, extravagant, enthusiastic, and disorderly, while low scorers are indifferent, reflective, frugal, detached, orderly, and regimented [4]. The T allele of the GCH1 polymorphism was related to lower luciferase activity in transfected chromaffin cells [22], while a GCH1 haplotype in strong linkage disequilibrium with this allele [8] was associated with lower GCH1 mRNA levels and reduced BH4 levels in vascular and leucocytes [22,20]. These findings lead to the assumption that the subjects with the T allele of the GCH1 polymorphism have lower levels of dopamine, serotonin, and NO in brain. Thus, the present results suggest that males with the GCH1 genotype predictive of lower levels of these neurotransmitters have lower scores of novelty seeking.

![Fig. 1. Involvement of GCH1 in biosynthesis of dopamine, serotonin, and nitric oxide. GCH1, GTP cyclohydrolase 1; PTPS, 6-pyruvoyl-tetrahydropterin synthase; SR, sepiapterin reductase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; PAH, phenylalanine hydroxylase; NOS, nitric oxide synthase.](image-url)
It has been suggested that dopaminergic neurotransmission is involved in novelty seeking [4]. Specifically, the relationship between lower novelty seeking and higher synaptic dopamine levels was supported by the genetic association studies on the tyrosine hydroxylase [16] and monoamine oxidase A [19] polymorphisms. Meanwhile, Reif et al. [15] showed that the males with the NO synthase genotype predictive of higher NO levels exhibited lower scores of excitement seeking of the NEO personality questionnaire [7], which correspond to lower scores of novelty seeking of the TCI [4]. These studies showing that either low dopamine or NO levels induce higher scores of novelty seeking are opposite to the present results. Since there have been no studies examining the effects of the GCH1 polymorphism on these neurotransmitter levels in brain, we cannot specify the exact mechanism. However, one plausible possibility is that lower levels of dopamine, NO, and serotonin caused by the GCH1 polymorphism may induce low scores of novelty seeking. In fact, it has been reported that NO exerts a negative control over the levels of dopamine and serotonin [21], while serotonin counteracts dopaminergic transmission by negative feedback [6]. Thus, it is possible that these interactions among dopamine, NO, and serotonin may be implicated in the results observed in the present study.

The significant association between the GCH1 polymorphism and novelty seeking was observed only in males, and none of the TCI dimensions was affected by the GCH1 polymorphism in females. It was reported that expression levels of GCH1 mRNA in brain monoaminergic neurons were higher in male mice than in female mice [18], probably because of modulating effects of sex hormones on GCH1 expression [17]. Therefore, the sex-specificity observed in the present study may be due to the effects of sex hormones on the regulation of GCH1 activity.

In conclusion, the present study suggests that the C>243T polymorphism in the GCH1 gene affects the personality trait of novelty seeking in males.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References


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Table 1
Effects of the GCH1 polymorphism and gender on TCI dimension scores.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Novelty seeking</th>
<th>Harm avoidance</th>
<th>Reward dependence</th>
<th>Persistence</th>
<th>Self-directedness</th>
<th>Cooperativeness</th>
<th>Self-transcendence</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>185</td>
<td>22.9 ± 4.8</td>
<td>18.1 ± 6.2</td>
<td>15.5 ± 3.7</td>
<td>4.3 ± 1.7</td>
<td>28.9 ± 6.1</td>
<td>28.1 ± 4.9</td>
</tr>
<tr>
<td>C/T</td>
<td>237</td>
<td>21.4 ± 5.1</td>
<td>18.1 ± 6.1</td>
<td>15.8 ± 3.6</td>
<td>4.4 ± 1.8</td>
<td>29.6 ± 6.2</td>
<td>27.8 ± 5.6</td>
</tr>
<tr>
<td>T/T</td>
<td>75</td>
<td>21.0 ± 4.6</td>
<td>18.2 ± 5.5</td>
<td>15.7 ± 3.9</td>
<td>4.7 ± 1.8</td>
<td>29.4 ± 6.0</td>
<td>27.5 ± 5.3</td>
</tr>
</tbody>
</table>

Gender, Genotype, Genotype x Gender main effects, F

- Gender: 0.801, p=0.133
- Interaction: 18.857b, p=0.002

Fig. 2. Post-hoc analysis of the effects of the GCH1 genotypes on the scores of novelty seeking in males and females. Error bars indicate SD of the mean.