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MICRO REPORT

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# Comprehensive behavioral analysis and quantification of brain free amino acids of C57BL/6J congenic mice carrying the 1473G allele in tryptophan hydroxylase-2

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## Abstract

**Aim:** Tryptophan hydroxylase 2 (Tph2) is a rate-limiting enzyme for the biosynthesis of 5-hydroxytryptamine (5-HT, serotonin). Previous studies have reported that C1473G polymorphism of the murine *Tph2* gene leads to decreased 5-HT levels in the brain and abnormal behavioral phenotypes, such as impaired anxiety- and depression-like behaviors. In this study, to confirm the effect of the C1473G polymorphism on mouse phenotypes, we conducted a comprehensive battery of behavioral tests and measured the amounts of brain free amino acids involved in the production of 5-HT.

**Methods:** We obtained C57BL/6J congenic mice that were homozygous for the 1473G allele of *Tph2* (1473G) and subjected them and their wild-type littermates (1473C) to a battery of behavioral tests. Using reverse-phase high-performance liquid chromatography (HPLC), we measured the amounts of free amino acids in the 5-HT and epinephrine synthetic/metabolic pathways in the frontal cortex, hippocampus, striatum, and midbrain.

**Results:** We failed to detect significant differences between genotypes in depression-like behaviors, anxiety-like behaviors, social behaviors, sensorimotor gaiting, or learning and memory, while 1473G mice exhibited a nominally significant impairment in gait analysis, which failed to reach study-wide significance. In the HPLC analysis, there were no significant differences in the amounts of 5-HT, dopamine, norepinephrine, and epinephrine in the frontal cortex, hippocampus, striatum, and midbrain.

**Conclusion:** Our findings do not support the idea that congenic C57BL/6J mice carrying the 1473G allele may represent an animal model of mood disorder under normal conditions without stress.

KEYWORDS

5-HT, comprehensive behavioral test battery, depression-like behavior, tryptophan hydroxylase 2

Koshimizu and Hirata contributed equally to this work.

## MAIN TEXT

Tryptophan hydroxylase (Tph) is a rate-limiting enzyme in 5-hydroxytryptamine (5-HT, serotonin) biosynthesis,[1] and to date, two isoforms of Tph have been identified in mammals. Tph1 is mainly expressed in the periphery, and Tph2 is preferentially expressed in the brain.[2] Zhang et al. reported that C1473G polymorphism exists in the mouse *Tph2* gene, and the mutant mice show decreased synthesis of 5-HT in the brain.[3] Previous studies demonstrated that mice homozygous for the 1473G allele of *Tph2* (1473G) exhibit abnormal behavioral phenotypes, such as impaired anxiety- and depression-like behaviors.[4,5] In contrast, other groups have failed to detect these abnormal behaviors in 1473G mice.[6–10] The biological significance of C1473G polymorphism remains controversial. C1473G polymorphism is reported to lead to a proline to arginine substitution and disturbance of 5-HT synthesis.[3] This sequence alteration and the amount of 5-HT differ depending on the mouse line.[3,4,8,11] Some mouse lines, including C57BL/6 and 129X1/SvJ, are homozygous for the 1473C allele (1473C), but other lines, such as BALB/c and DBA/2J, are homozygous for the 1473G allele (Table S1), causing decreased 5-HT synthesis compared to 1473C mice.[3,12] The objective of the present study was to further investigate the functional significance of C1473G polymorphism in mice.

We prepared congenic C57BL/6J (B6J) mice using a backcrossing breeding strategy.[4,7] In brief, heterozygous mice were created from hybrids between Balb/c AJc1 and B6J strains, which are homozygous for the 1473G and 1473C allele, respectively. After six successive backcrossings of heterozygous mice with the B6J strain, the heterozygous backcrosses were intercrossed to generate congenic B6J mice homozygous for the 1473G and 1473C allele. We subjected those 1473G and 1473C mice to a comprehensive behavioral test battery that included the wire hang, grip strength, rotarod, hot plate, gait analysis, tail suspension, Porsolt forced swim, open field, light/dark transition, elevated plus maze, social interaction, sociability and social novelty preference, startle response/prepulse inhibition (PPI), and fear conditioning tests, as previously described.[13–16] All behavioral tests were carried out with male mice that were at least 19 weeks old at the start of testing (Table S3). The behavioral results are summarized in Table 1. There were no significant differences between the genotypes in physical characteristics or on the wire hang, grip strength, rotarod, and hot plate tests. In the gait analysis, 1473G mice exhibited nominally significant impairments in the stance width of the hind paws and the step angles of the front paws, but these results failed to reach study-wide significance. None of the indices of the tail suspension and Porsolt forced swim tests (Figure S1) showed significant differences between the genotypes. No genotype-specific differences were observed in the open field (Figure S2), light/dark transition, and elevated plus-maze tests. There were no significant genotype effects in the social interaction, sociability, and social novelty preference tests. In the startle response/PPI tests, 1473G mice displayed normal acoustic startle responses and sensorimotor gating. No obvious differences between the genotypes were detected in the fear conditioning tests (Figure S3).

We next quantified the amount of free amino acids that are involved in the 5-HT metabolic pathway (eg, 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA)) in the prefrontal cortex, hippocampus, striatum, and midbrain using reverse-phase high-performance liquid chromatography (HPLC), as previously described.[17] We also measured the amounts of free amino acids in the epinephrine (Epi) synthetic/metabolic pathway (eg, dopamine (DA), 3-methoxytyramine (3-MT), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), normetanephrine (NM), 4-hydroxy-3-methoxyphenylglycol (MHPG), and Epi). The quantitative results are summarized in Table S2. The amounts of free amino acids in the hippocampus, striatum, and midbrain did not significantly differ between the genotypes, while there was a non-significant tendency toward decreased 5-HT in the prefrontal cortex in 1473G mice.

There are inconsistencies in the biochemical phenotype of the *Tph2* 1473G allele-carrying mice among the present and previous studies. The present and a previous study failed to detect significant changes in the free amino acid (5-HT, 5-HTP, and 5-HIAA) level in the frontal cortex, hippocampus, stratum, or midbrain,[6] but other studies have reported significant genotype effects in some of these areas [3,4,8–11] (Table S1). No major changes were detected in the behavioral phenotype of the C57BL/6J mice carrying the 1473G allele in the present and some previous studies,[6–10] while a few previous studies have reported significant changes in depression-like and/or anxiety-like behavior [4,5] (Table S1). These inconsistencies may be due to differences in factors such as genetic background, flanking genes, age, exposure of the animals to stress, and/or experimental environments/conditions.

In conclusion, we failed to detect major differences in depression- and anxiety-like behaviors or levels of brain free amino acids in 1473G mice on a C57BL/6J genetic background, while 1473G mice exhibited nominally significant impairments in the gait analysis, which failed to reach study-wide significance. Under conditions without stress or drug administration, C57BL/6J mice homozygous for the *Tph2* 1473G allele displayed no significant behavioral or physiological phenotype, indicating that these congenic mice may not represent an animal model of mood disorder.

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**TABLE 1** Comprehensive behavioral battery in 1473G and 1473C mice

| Test | 1473G (n = 7) | 1473C (n = 7) | Genotype effect | |
|---|---|---|---|---|
| | | | *F*-value | *P*-value |
| Physical characteristics | | | | |
| Weight (g) | 31.743 (±0.566) | 31.786 (±0.635) | $F_{1,12}$ = 0.003 | 0.9606 |
| Body temperature (°C) | 37.100 (±0.298) | 37.299 (±0.252) | $F_{1,12}$ = 0.109 | 0.7474 |
| Neurological screen and neuromuscular strength test | | | | |
| Grip strength (N) | 0.893 (±0.037) | 0.929 (±0.054) | $F_{1,12}$ = 0.295 | 0.5968 |
| Wire hang (% falling within 60 s) | 48.143 (±5.230) | 52.000 (±6.904) | $F_{1,12}$ = 0.198 | 0.664 |
| Rotarod test | | | | |
| Latency to fall (s) | 148.143 (±17.358) | 179.452 (±11.009) | $F_{1,12}$ = 2.32 | 0.1536 |
| Hot plate test | | | | |
| Latency (s) | 3.014 (±0.122) | 3.457 (±0.473) | $F_{1,12}$ = 0.822 | 0.3826 |
| Gait analysis | | | | |
| Stance width (cm) | | | | |
| Front | 1.421 (±0.041) | 1.357 (±0.048) | $F_{1,12}$ = 1.043 | 0.3273 |
| Hind | 2.029 (±0.036) | 1.900 (±0.038) | $F_{1,12}$ = 6.075 | 0.0298 |
| Step angles (°) | | | | |
| Front | 68.914 (±2.171) | 59.900 (±3.393) | $F_{1,12}$ = 5.008 | 0.045 |
| Hind | 49.850 (±3.301) | 60.343 (±5.215) | $F_{1,12}$ = 2.891 | 0.1148 |
| Tail suspension test | | | | |
| Immobility (%) | 28.901 (±7.352) | 14.549 (±2.925) | $F_{1,12}$ = 3.29 | 0.0948 |
| Porsolt forced swim test | | | | |
| Immobility (%) | | | | |
| Day 1 | 57.28 (±3.447) | 56.667 (±2.559) | $F_{1,12}$ = 0.02 | 0.8889 |
| Day 2 | 62.839 (±5.179) | 58.45 (±5.755) | $F_{1,12}$ = 0.321 | 0.5813 |
| Distance traveled (cm) | | | | |
| Day 1 | 83.629 (±3.431) | 87.823 (±3.525) | $F_{1,12}$ = 0.727 | 0.4106 |
| Day 2 | 68.351 (±4.33) | 83.61 (±6.752) | $F_{1,12}$ = 3.619 | 0.0814 |
| Open field test | | | | |
| Distance traveled (cm) | 571.798 (±83.344) | 623.577 (±55.978) | $F_{1,12}$ = 0.266 | 0.6154 |
| Number of vertical activities | 61.905 (±7.868) | 72.232 (±5.937) | $F_{1,12}$ = 1.098 | 0.3154 |
| Center time (s) | 52.904 (±10.598) | 41.015 (±5.684) | $F_{1,12}$ = 0.977 | 0.3424 |
| Stereotypic counts | 653.768 (±48.016) | 621.137 (±66.817) | $F_{1,12}$ = 0.157 | 0.6986 |
| Light/dark transition test | | | | |
| Stay time in light compartment (s) | 193.929 (±18.927) | 190.143 (±14.931) | $F_{1,12}$ = 0.025 | 0.8778 |
| Number of transitions | 20.571 (±3.108) | 21.429 (±1.837) | $F_{1,12}$ = 0.056 | 0.8163 |
| Elevated plus-maze test | | | | |
| Open arms entries per total entries (%) | 30.292 (±3.696) | 31.19 (±4.417) | $F_{1,12}$ = 0.024 | 0.8787 |
| Stay time ratio on open arms (%) | 13.667 (±3.321) | 13.69 (±2.641) | $F_{1,12}$ < 0.0001 | 0.9956 |
| Social interaction test | | | | |
| Total duration of contact (s) | 75.333 (±5.487) | 69.333 (±8.098) | *F*1,4 = 0.376 | 0.5728 |
| Number of contacts | 55.667 (±8.511) | 56.333 (±2.028) | *F*1,4 = 0.006 | 0.9429 |
| Total duration of active contacts (s) | 17.567 (±2.969) | 17.567 (±0.696) | *F*1,4 = 0 | 1 |
| Mean duration per contacts | 1.433 (±0.145) | 1.233 (±0.133) | *F*1,4 = 1.029 | 0.3679 |
| Distance traveled (cm) | 3847.5 (±268.099) | 430.667 (±317.643) | *F*1,4 = 1.968 | 0.2333 |

*(Continues)*

**TABLE 1** (Continued)

| Test | 1473G (n = 7) | 1473C (n = 7) | Genotype effect | |
|---|---|---|---|---|
| | | | *F*-value | *P*-value |
| Sociability and social novelty preference test | | | | |
| Sociability test | | | | |
| Empty side | 97.571 (±13.811) | 95.0 (±10.207) | $F_{1,12}$ = 0.022 | 0.8835 |
| Time spent around cage (s) | | | | |
| Stranger side | 143.143 (±20.036) | 123.286 (±13.852) | $F_{1,12}$ = 0.665 | 0.4308 |
| Social novelty preference test | | | | |
| Familiar side | 127.714 (±20.757) | 121.429 (±20.903) | $F_{1,12}$ = 0.046 | 0.8346 |
| Time spent around cage (s) | | | | |
| Stranger side | 152.571 (±20.752) | 135.143 (±17.07) | $F_{1,12}$ = 0.421 | 0.5288 |
| Startle response/prepulse inhibition test | | | | |
| Startle amplitude | | | | |
| 110 dB | 0.614 (±0.077) | 0.844 (±0.203) | $F_{1,12}$ = 0.842 | 0.377 |
| 120 dB | 1.219 (±0.215) | 1.397 (±0.158) | | |
| Prepulse inhibition (%) (Prepulse sound level/startle) | | | | |
| 74/110 dB | 28.393 (±14.088) | 28.81 (±11.223) | $F_{1,12}$ = 0.099 | 0.7579 |
| 78/110 dB | 48.843 (±10.047) | 35.283 (±28.902) | | |
| 74/120 dB | 21.15 (±8.938) | 10.787 (±10.207) | $F_{1,12}$ = 0.805 | 0.3873 |
| 78/120 dB | 47.225 (±9.853) | 34.074 (±13.286) | | |
| Fear conditioning test | | | | |
| Freezing (%) | | | | |
| Conditioning | 27.229 (±3.602) | 26.452 (±4.429) | $F_{1,12}$ = 0.019 | 0.894 |
| Context testing | 26.514 (±8.652) | 34.697 (±7.528) | $F_{1,12}$ = 0.509 | 0.4892 |
| Cued testing with altered context | 58.331 (±4.568) | 54.807 (±3.57) | $F_{1,12}$ = 0.369 | 0.5546 |

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## CONFLICT OF INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interests.

## AUTHORS' CONTRIBUTIONS

TM was responsible for the original conception and overall design of the research. KTa and KTo established the congenic mice and performed the comprehensive behavioral test battery. TI and SF conducted the quantification of the amino acids. HK, NH, KTa, KTo, TI, SF, and TM analyzed the data. HK, NH, and TM wrote the manuscript. All authors read and approved the final manuscript.

## DATA REPOSITORY

Raw data on the behavioral tests and the information about each mouse are accessible on the public database "Mouse Phenotype Database" (http://www.mouse-phenotype.org/).

## ANIMAL STUDIES

All behavioral testing procedures were approved by the Institutional Animal Care and Use Committee of Graduate School of Medicine of Kyoto University and Fujita Health University.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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