doi 10.18699/bgrs2024-7.1-14

The C886T mutation in the *Th* gene reduces the activity of tyrosine hydroxylase in the brain of mice

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Key words: tyrosine hydroxylase; C886T mutation; activity; expression; brain; mice

Motivation and Aim: The dopaminergic (DA) system in the brain plays a pivotal role in regulating the nervous system, endocrine glands, and both adaptive and pathological behaviors [1]. DA synthesis in the brain occurs in two steps from the amino acid L-tyrosine: first, the enzyme tyrosine hydroxylase (TH) converts L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), and then, the enzyme aromatic amino acid decarboxylase converts L-DOPA to DA. The hydroxylation of L-tyrosine is the key factor determining DA levels in the brain. Some mutations in the human *TH* gene are associated with childhood parkinsonism, an increased risk of Parkinson's disease [2], dystonia [3], and bipolar disorders [4]. Understanding the molecular events associated with mutations in the *TH* gene and their effects on the nervous system, motor function, and psychological functions of humans is restricted due to social and ethical constraints. Therefore, the modeling of molecular events caused by mutations in laboratory rodents becomes highly relevant. The Ensembl genome database (https://www.ensembl.org/ index.html) contains information on 21 SNPs in the mouse *Th* gene, leading to amino acid substitutions in the TH molecule. Among these, only one SNP, the C886T mutation resulting in the R278H substitution in the TH molecule, is identified in widely used laboratory mouse strains. This study aims to investigate the impact of the C886T mutation in the *Th* gene on the TH activity in the mouse midbrain, the region containing the DA neuron bodies.
Methods and Algorithms: This study is carried out on adult males of C57BL/6 (886T, $n = 6$), DBA/2 (886T, $n = 6$), CAST (886C, $n = 5$) lines as well as males and females of F2 segregating intercrosses between C57BL/6 and CAST mice ($n = 42$). TH activity is assayed by HPLC technique and quantified in picomoles (pmol) of L-DOPA synthesized per minute per milligram of protein. Genotyping of the 886T and 886C alleles was accomplished through quantitative real-time qPCR technique. *Th* gene mRNA and TH protein levels are assayed by qPCR and western blot analysis, respectively.
Results: Mice from the three strains exhibited differential TH activity in the midbrain ($F(2,14) = 21.68$, $p < 0.001$). CAST mice displayed significantly higher TH activity compared to C57BL/6 ($p < 0.001$) and DBA/2 ($p < 0.001$) mice. However, no interstrain differences in *Th* gene mRNA levels ($F(2,13) = 2.67$, $p = 0.11$,) or TH protein levels ($F(2,14) = 1.23$, $p = 0.32$) in the midbrain were observed (Fig. 1).
Distribution of TT, TC and CC genotypes among F2 intercross mice was consistent with the expected 1:2:1 ratio ($\chi2(1) = 0.805$, $p > 0.05$) (Table 1).

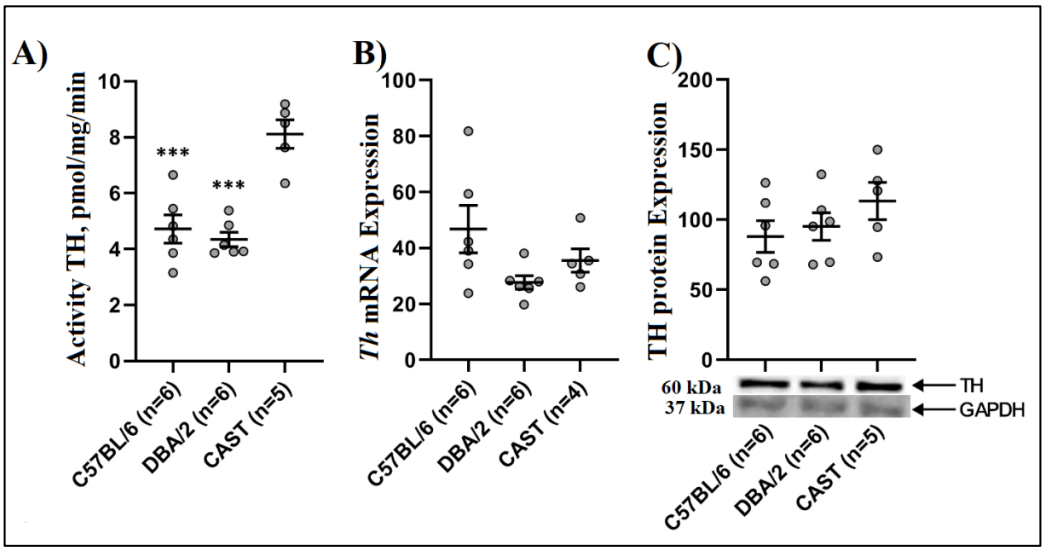

Fig. 1. TH Activity (A), *Th* gene mRNA level (B), and TH protein level (C) in the midbrain of mice of C57BL/6 (TT), DBA/2 (TT), and (CAST) (CC) lines. Individual values are shown with means ± SEM. *Th* gene expression is normalized to *Polr2a* gene expression, and TH protein levels are normalized to GAPDH protein levels. ***$p < 0.001$ vs. CAST. Between-line differences were evaluated using one-way ANOVA

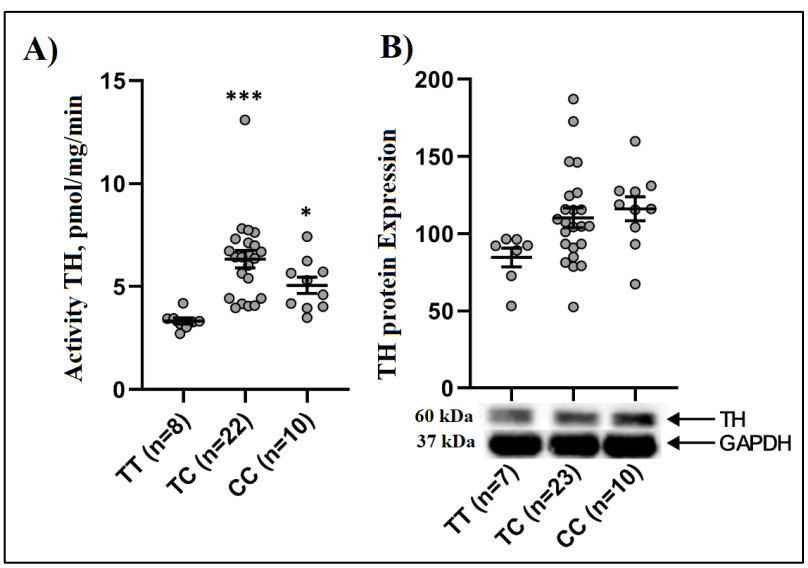

Fig. 2. TH Activity (A) and Protein Level (B) in the midbrain of F2 intercrosses with TT, TC, and CC genotypes. Data for males and females are pooled. Individual values are shown with means ± SEM. The TH protein level is normalized to the GAPDH protein level. *$p < 0.05$, **$p < 0.001$ vs. TT. F2 intercrosses were analyzed using two-way ANOVA. Fisher's LSD method was used for intergroup comparisons

Table 1. Distribution of TT, TC, and CC genotypes among the male and female F2 intercross offspring, obtained from the mating of F1 hybrids (C57BL/6 x CAST)

Genotype	Males	Females	Males + Females
TT	5	3	8
TC	13	10	23
CC	5	6	11

Among F2 intercross mice, TH activity was significantly lower in individuals with the TT genotype compared to TC ($p < 0.001$) and CC ($p = 0.02$) genotypes (Fig. 2A), while TH protein levels showed no significant differences between genotypes ($F(2,38) = 2.22$, $p = 0.12$, Fig. 2B).

Conclusion: This pilot study clearly establishes the significance of the C886T polymorphism as a critical determinant of TH activity in the mouse brain. Notably, being the first naturally occurring common mutation in the mouse *Th* gene shown to impact enzyme activity, this mutation opens up promising avenues for further experimental modeling to explore its effects on physiological functions, both in normal and pathological conditions.

Funding: The study is supported by Russian Science Foundation, grant No. 24-15-00078.

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