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A FUNCTIONAL POLYMORPHISM IN THE MOUSE *TH* GENE ALTERS TYROSINE HYDROXYLASE ACTIVITY AND MIDBRAIN DOPAMINE LEVELS *

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Abstract

A C886T (R278H) polymorphism in the mouse *Th* gene reduces tyrosine hydroxylase enzymatic activity and dopamine levels in the midbrain without altering Th mRNA or protein expression. Computational predictions, allele-specific genotyping, and HPLC-based biochemical assays support a functional impact of this variant on dopamine biosynthesis.

The dopaminergic (DA) system plays a pivotal role in regulating the nervous system, endocrine function, and both adaptive and pathological behaviors [1]. Catecholamine biosynthesis begins with the amino acid L-tyrosine: tyrosine hydroxylase (TH) catalyzes its conversion to L-3,4-dihydroxyphenylalanine (L-DOPA), which is then decarboxylated to dopamine (DA) by aromatic amino acid decarboxylase. DA can be further metabolized to norepinephrine (NE) by dopamine β-hydroxylase, and subsequently to epinephrine (Epi) by phenylethanolamine N-methyltransferase. Thus, TH-mediated hydroxylation of L-tyrosine represents the rate-limiting step in the biosynthesis of DA, NE, and Epi in both central nervous system neurons and adrenal chromaffin cells. Mutations in the human TH gene have been linked to childhood parkinsonism [2], dystonia [3], and bipolar disorder [4], underscoring its pathophysiological relevance.

The Ensembl genome database (https://www.ensembl.org) lists 21 nonsynonymous single nucleotide polymorphisms (SNPs) in the mouse *Th* gene. To assess the potential functional significance of these SNPs, we evaluated their predicted impact on TH protein stability using three in silico tools: I-Mutant, MUpro, and SAAFEC-SEQ. These tools generate ΔΔG values, which quantify the change in protein stability resulting from single-point mutations. Mutations with $\Delta\Delta G < -0.5$ were selected for further analysis.

Allele-specific primers were designed to identify polymorphisms distinguishing CAST/EiJ and C57BL/6 mouse strains. One such SNP, C886T (R278H), was identified: C57BL/6 mice carry the 886T allele, while CAST/EiJ mice carry the 886C allele.

This study investigates the functional consequences of the C886T mutation on TH activity in the mouse midbrain, where dopaminergic neuron cell bodies are located.

Male C57BL/6 (886T/T), CAST/EiJ (886C/C) were used. F2 intercrosses were generated by crossing C57BL/6 and CAST/EiJ mice. To introgress the 886C allele into the C57BL/6 background, ten successive backcrosses were performed. Intercrossing of BC10×BC10 animals produced mice of all three genotypes (CC, TC, TT). Genotyping was performed using allele-specific qPCR. TH enzymatic activity was measured by HPLC-based quantification of L-DOPA accumulation. Dopamine and its metabolites — homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) — were also quantified by HPLC in BC10×BC10 hybrids. *Th* mRNA and TH protein expression were analyzed by quantitative PCR and Western blot, respectively.

TH activity was significantly higher in CAST/EiJ (886C/C) midbrains compared to C57BL/6 (886T/T) mice ($p < 0.001$). However, no differences were observed in *Th* mRNA ($p = 0.11$) or protein levels ($p = 0.32$). In F2 intercrosses, TT mice exhibited significantly lower TH activity than CC or TC genotypes ($p < 0.05$), with no corresponding differenc-

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es in TH protein expression (p = 0.12). BC10×BC10 hybrids displayed a genotype-dependent gradient in TH activity: CC > TC > TT (p < 0.001). Midbrain DA levels were significantly higher in CC mice compared to TT and TC (p < 0.05), while HVA and DOPAC levels were not significantly different across genotypes.

The 886T allele lowers TH enzymatic activity and midbrain dopamine levels independently of transcript or protein expression changes, pointing to a functional (rather than transcriptional) defect. We propose that this SNP impairs TH's catalytic efficiency, stability, or post-translational regulation. Recombinant expression of the C886T variant would definitively test this hypothesis, clarifying its direct role in dopaminergic dysfunction.

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