University of Szeged

Albert Szent-Györgyi Medical School

Doctoral School of Clinical Medicine

Department of Neurology

Tryptophan-Kynurenine Metabolic Remodeling and Complementary Pathways in Kynurenine Aminotransferase II Knockout Mice with Relevance to Neuropsychiatric Phenotypes

Ph.D. Thesis

Ágnes Szabó

Supervisor(s):

László Vécsei M.D., Ph.D., D.Sc.

Masaru Tanaka M.D., Ph.D.

Szeged

2026

Original Publication directly related to the Ph.D. thesis:

I. **Szabó Á.,** Galla Zs., Spekker E., Szűcs M., Martos D., Takeda K., Ozaki K., Inoue H., Yamamoto S., Toldi J., Ono E., Vécsei L., Tanaka M. *Oxidative and Excitatory Neurotoxic Stresses in CRISPR/Cas9-Induced Kynurenine Aminotransferase Knockout Mice: A Novel Model for Despair-Based Depression and Post-Traumatic Stress Disorder.* **Frontiers in Bioscience Landmark** 2025, 30(1): 25706. doi: 10.31083/FBL25706 (**original paper, Q2, IF: 3.100 (2025))**

II. **Szabó Á.,** Galla Zs., Spekker E., Martos D., Szűcs M., Fejes-Szabó A., Fehér Á., Takeda K., Ozaki K., Inoue H., Yamamoto S., Monostori P., Toldi J., Ono E., Vécsei L., Tanaka M. *Behavioral Balance in Tryptophan Turmoil: Regional Metabolic Rewiring in Kynurenine Aminotransferase II Knockout Mice.* **Cells** 2025, 14, 1711. doi: 10.3390/cells14211711 (**original paper, Q1, IF: 5.200 (2025))**

Quartile ranking of the publication directly related to the thesis: 1 Q1 + 1 Q2
Cumulative impact factor of the publications directly related to the thesis: 8.300

Publications not directly related to the Ph.D. thesis:

I. Tanaka M., **Szabó Á.,** Lőrinczi B., Szatmári I., Fülöp F., Vécsei L. Antidepressant-like Effects of Kynurenic Acid Analogues. **Proceedings of 1st International Electronic Conference on Biomedicine** 2021, 10301, 8 p. doi: 10.3390/ECB2021-10301

II. Tanaka M., Tóth F., Polyák H., **Szabó Á.,** Mándi Y., Vécsei L. Immune Influencers in Action: Metabolites and Enzymes of the Tryptophan-Kynurenine Metabolic Pathway. **Biomedicines** 2021, 9, 734. doi: 10.3390/biomedicines9070734 **(Q1, IF: 4.757 (2021))**

III. Tanaka M., Török N., Tóth F., **Szabó Á.,** Vécsei L. *Co-Players in Chronic Pain: Neuroinflammation and the Tryptophan-Kynurenine Metabolic Pathway.* **Biomedicines 2021,** 9, 897. doi: 10.3390/biomedicines9080897 **(Q1, IF: 4.757 (2021))**

IV. Spekker E., Tanaka M., **Szabó Á.,** Vécsei L. *Neurogenic Inflammation: The Participant in Migraine and Recent Advancements in Translational Research.* **Biomedicines** 2021, 10, 76. doi: 10.3390/biomedicines10010076 **(Q1, IF: 4.757 (2021))**

V. Tanaka M., Spekker E., **Szabó Á.,** Polyák H., Vécsei L. *Modelling the neurodevelopmental pathogenesis in neuropsychiatric disorders. Bioactive kynurenines and their analogues as neuroprotective agents—in celebration of 80th birthday of Professor Peter Riederer.* **Journal of Neural Transmission** 2022. doi: 10.1007/s00702-022-02513-5 **(Q2, IF: 3.300 (2022))**

VI. Tanaka M., **Szabó Á.,** Spekker E., Polyák H., Tóth F., Vécsei L. *Mitochondrial Impairment: A Common Motif in Neuropsychiatric Presentation? The Link to the Tryptophan–Kynurenine Metabolic System.* **Cells** 2022, 11, 2607. doi: 10.3390/cells11162607 **(Q1, IF: 6.000 (2022))**

VII. Tanaka M., **Szabó Á.,** Vécsei L. *Integrating Armchair, Bench, and Bedside Research for Behavioral Neurology and Neuropsychiatry: Editorial.* **Biomedicines** 2022, 10, 2999. doi: 10.3390/biomedicines10122999 **(Q1, IF: 4.700 (2022))**

VIII. Tajti J., Szok D., Csáti A., **Szabó Á.,** Tanaka M., Vécsei L. *Exploring Novel Therapeutic Targets in the Common Pathogenic Factors in Migraine and Neuropathic Pain.* **International Journal of Molecular Sciences** 2023, 24, 4114. doi: 10.3390/ijms24044114 **(Q1, IF: 4.900 (2023))**

IX. Polyák H., Galla Zs., Nánási N., Cseh E.K., Rajda C., Veres G., Spekker E., **Szabó Á.,** Klivényi P., Tanaka M., Vécsei L. *The Tryptophan-Kynurenine Metabolic System Is Suppressed in Cuprizone-Induced Model of Demyelination Simulating Progressive Multiple Sclerosis.* **Biomedicines** 2023, 11, 945. doi: 10.3390/biomedicines11030945 **(Q1, IF: 3.900 (2023))**

X. Tanaka M., **Szabó Á.,** Vécsei L. *Preclinical modeling in depression and anxiety: Current challenges and future research directions.* **Advances in Clinical and Experimental Medicine** 2023, 32(5). doi: 10.17219/acem/165944 **(Q2, IF: 2.100 (2023))**

XI. Tanaka M., **Szabó Á.,** Körtési T., Szok D., Tajti J., Vécsei L. *From CGRP to PACAP, VIP, and Beyond: Unraveling the Next Chapters in Migraine Treatment.* **Cells** 2023, 12, 2649. doi: 10.3390/cells12222649 **(Q1, IF: 5.100 (2023))**

XII. Tanaka M., **Szabó Á.,** Vécsei L., Giménez-Llort L. *Emerging Translational Research in Neurological and Psychiatric Diseases: From In Vitro to In Vivo Models.* **International Journal of Molecular Sciences** 2023, 24, 15739.doi: 10.3390/ijms242115739 **(Q1, IF: 4.900 (2023))**

XIII. Tanaka M., **Szabó Á.,** Vécsei L. *Redefining Roles: A Paradigm Shift in Tryptophan–Kynurenine Metabolism for Innovative Clinical Applications.* **International Journal of Molecular Sciences** 2024, 25, 12767. doi: 10.3390/ijms252312767 **(Q1, IF: 4.900 (2024))**

XIV. Juhász L., Spisák K., Szolnoki B.Zs., Nászai A., **Szabó Á.,** Rutai A., Tallósy Sz.P., Szabó A., Toldi J., Tanaka M., Takeda K., Ozaki K., Inoue H., Yamamoto S., Ono E., Boros M., Kaszaki J., Vécsei L. *The Power Struggle: Kynurenine Pathway Enzyme Knockouts and Brain Mitochondrial Respiration.* **Journal of Neurochemistry** 2025, 169:e70075. doi: 10.1111/jnc.70075 **(Q1, IF: 3.700 (2024))**

Quartile ranking of publications not directly related to the thesis: 11 Q1 + 2 Q2
Cumulative impact factor of publications not directly related to the thesis: 57.771
Total quartile ranking: 12 Q1 + 3 Q2
Total impact factor: 66.071

List of abbreviations

3CT	three chamber test	OBAT	object-based attention test
3-HAA	3-hydroxyanthranilic acid	OFT	open-field test
3-HK	3-hydroxykynurenine	OSI	oxidative stress index
5-HT	5-hydroxytryptamine/serotonin	PAT	passive avoidance test
AA	anthranilic acid	SD	standard deviation
AADC	aromatic L-amino acid decarboxylase	STEM	brainstem
ALDH	aldehyde dehydrogenase	STR	striatum
ANOVA	analysis of variance	TMO	tryptophan-2-monooxygenase
CER	cerebellum	Trp	tryptophan
CTX	cortex	TST	tail suspension test
DA	dopamine	Tyr	tyrosine
DOPAC	3,4-dihydroxyphenylacetic acid	UHPLC-MS/MS	ultra-high-performance liquid chromatography coupled with tandem mass
EI	excitotoxicity index	WT	wild-type
EPM	elevated plus maze	XA	xanthurenic acid
FST	forced swim test		
HIPP	hippocampus		
IAA	indole-3-acetic acid		
KAT II	kynurenine aminotransferase II		
kat2$^{-/-}$	kynurenine aminotransferase II knockout strain		
KATs	kynurenine aminotransferases		
KMO	kynurenine 3-monooxygenase		
KYN	kynurenine		
KYNA	kynurenic acid		
KYNU	kynureninase		
LDB	light dark box		
MAOs	monoamine oxidases		
NORT	novel object recognition test		

Introduction

Tryptophan (Trp) is an essential amino acid that serves as a central precursor for several interconnected metabolic pathways. The vast majority of Trp is metabolized through the kynurenine (KYN) pathway, during which the energy-carrying cofactor nicotinamide adenine dinucleotide is synthesized along with multiple biologically active intermediates. KYN can be diverted into several metabolically and functionally distinct branches, among which the formation of kynurenic acid (KYNA) is particularly important. KYNA is produced via transamination of KYN catalyzed by kynurenine aminotransferases (KATs). Under physiological conditions, kynurenine aminotransferase II (KAT II) is the predominant isoform in the brain.

Trp metabolism also proceeds through additional pathways. Despite accounting for a relatively small proportion of total Trp utilization, the serotonin (5-HT) pathway plays a fundamental role in mood regulation and cognitive processes. The indole-pyruvate pathway, linked to the gut–brain axis, influences immunological processes through microbiota-derived indole metabolites. In addition, Trp metabolism is functionally connected to the tyrosine (Tyr)–dopamine (DA) system via shared cofactors and redox-dependent mechanisms.

Metabolites of the KYN pathway can be neuroprotective or neurotoxic, pro-oxidant or antioxidant, and immunomodulatory, with effects that are strongly context- and concentration-dependent. Disruption of metabolic balance has been described in a wide range of neurological and psychiatric disorders; however, it remains unclear whether these alterations are consequences of disease processes or actively contribute to their development.

Investigation of these mechanisms is greatly facilitated by genetically modified animal models. The KAT II knockout mouse model (*kat2*$^{-/-}$), generated by CRISPR/Cas9-mediated deletion of the *aadat* gene and forming the basis of the present work, provides an opportunity to examine how the absence of KAT II affects KYNA formation, the balance of Trp-associated metabolic pathways, and the resulting behavioral and metabolic consequences.

Aims

I. Following the generation of the *kat2*$^{-/-}$ mouse model, our first aim was to verify the genetic background of the animal colony prior to each experimental series, to confirm the presence of the gene knockout and ensure genetic integrity.

II. Subsequently, we aimed to comprehensively investigate the effects of reduced KAT II activity on the metabolic balance of Trp and its associated degradation pathways.

III. As part of the study, we aimed to perform baseline phenotypic characterization of *kat2*$^{-/-}$ mice using standardized methods to identify potential general, neurological, or sensorimotor alterations.

IV. Finally, we aimed to characterize the behavior of *kat2*$^{-/-}$ mice to determine how gene deletion affecting Trp metabolism influences distinct behavioral domains.

Materials and methods

Animals

C57BL/6N wild-type (WT) mice were obtained from Charles River Laboratories (Germany), while the *kat2*$^{-/-}$ mouse line was provided by our collaboration partners at Kyushu University (Fukuoka, Japan). Animals were housed under specific pathogen-free conditions in the animal facility of the Department of Neurology, University of Szeged, in polycarbonate cages (530 cm² floor area; 4–5 animals per cage). Environmental parameters were controlled (24 ± 1 °C, 45–55% relative humidity, 12 h light–dark cycle). Mice had ad libitum access to standard laboratory chow and drinking water, and environmental enrichment was provided. The importation of genetically modified animals was authorized by the Department of Biodiversity and Gene Conservation of the Ministry of Agriculture (BGMF/37-5/2020). All experimental procedures complied with the Ethical Codex for Animal Experiments and were approved by the Institutional Animal Welfare Committee of the University of Szeged and the National Food Chain Safety Office (XI./84/2025; X./1008/2025). The protocol for animal care was approved by the European Communities Council Directive (2010/63/EU) and the Hungarian Health Committee (40/2013 (II.14.)). Animal health was regularly monitored throughout the experiments. Animals reaching predefined humane endpoints were removed from the study.

I. Verification of the Genetic Background of kat2$^{-/-}$ *Mice*

All animals were genotyped before the start of the experiments. Tail biopsies were collected under 2% isoflurane anesthesia with the application of local lidocaine. Approximately 3-mm-long tail samples were excised under sterile conditions and stored at −80 °C until further processing. Genomic DNA was isolated using a modified alkaline lysis protocol based on the HotSHOT method. DNA concentration and purity were assessed by spectrophotometry, after which samples were stored at −20 °C. Genotype determination was performed using fluorescence-based TaqMan allelic discrimination in a real-time PCR system. Each assay included negative controls as well as validated WT and *kat2*$^{-/-}$ reference samples. Samples that could not be unambiguously classified were reanalyzed.

II. Metabolic Profiling of Tryptophan Degradation Pathways

Eight-week-old male *kat2*$^{-/-}$ and WT mice (n = 10 per group) were included in the metabolomic analyses. Urine samples were collected prior to the initiation of anesthesia. For plasma collection, animals were anesthetized with 2% isoflurane, and blood was drawn from the left cardiac ventricle into tubes containing disodium ethylenediaminetetraacetate dihydrate. Plasma was separated by centrifugation. For the determination of central nervous system metabolite levels, animals were transcardially perfused, after which brains were removed and dissected into five regions: striatum (STR), cortex (CTX), hippocampus (HIPP), cerebellum (CER), and brainstem (STEM). All samples were stored at -80 °C until further use. Brain tissues were homogenized in ice-cold water by an ultrasonic sonication method. Targeted quantification of Trp–KYN, serotonergic, indole-pyruvate, and catecholaminergic metabolites, as well as selected cofactors, was performed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) based on validated multiplex protocols with stable isotope-labeled internal standards. Relative enzyme activities were estimated by calculating product-to-substrate ratios. To characterize functional shifts in metabolic balance, the oxidative stress index (OSI) and excitotoxicity index (EI) were calculated to provide integrated measures of alterations in KYN pathway function.

III. Baseline Phenotypic Characterization of kat2$^{-/-}$ Mice

Eight-week-old male *kat2*$^{-/-}$ and WT mice (n = 10 per group) underwent standardized baseline phenotypic assessment using the RIKEN-validated modified SHIRPA protocol. All evaluations were conducted between 08:00 and 12:00, and animals were transferred to the testing room one hour prior to assessment for acclimatization. The SHIRPA protocol provides a comprehensive overview of general health, neurological status, and sensorimotor function, enabling the identification of baseline abnormalities that could influence subsequent behavioral testing. The assessment encompassed neuromuscular, autonomic, reflexive, and sensorimotor parameters.

IV. Behavioral Characterization of kat2$^{-/-}$ Mice

Eight-week-old male *kat2*$^{-/-}$ and WT mice (n = 10–13 per group) were included in the behavioral assessments. All tests were conducted between 08:00 and 12:00, and animals were transferred to the experimental room one hour prior to testing to allow for acclimatization. Between individual animals, all apparatuses were cleaned with 70% ethanol to minimize olfactory confounding factors.

Spontaneous locomotor and exploratory activity, as well as center preference, were assessed using the open field test (OFT), while motor coordination and balance were evaluated with the rotarod test. Anxiety-like behavior was examined using the elevated plus maze (EPM) and light–dark box (LDB) tests. Depression-like behavior was assessed using the modified forced

swim test (FST) and the tail suspension test (TST). Cognitive functions were evaluated using the passive avoidance test (PAT), novel object recognition test (NORT), object-based attention test (OBAT), and the Y-maze test. Repetitive behavior was assessed using the marble burying test in relation to anxiety-like behavior and cognition. Social behavior, including sociability and preference for social novelty, was evaluated using the three-chamber social test (3CT).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 28.0. Data distribution was assessed prior to inferential testing using the Shapiro–Wilk test, complemented by inspection of Q–Q plots. Homogeneity of variances was evaluated where appropriate, and outliers were identified using statistical methods. Comparisons between *kat2*$^{-/-}$ and WT groups were conducted using independent-samples t-tests for normally distributed data, while the nonparametric Mann–Whitney U test was applied when normality assumptions were violated. Phenotypic data obtained using the SHIRPA protocol were analyzed using one-way analysis of variance (ANOVA), with Tamhane post hoc tests applied when necessary. For behavioral paradigms involving multiple dependent variables or repeated measures, mixed-design ANOVA was employed. All statistical tests were two-tailed, and statistical significance was set at $p < 0.05$. Data are presented as mean ± standard deviation (SD).

Results

I. Verification of the Genetic Background of kat2$^{-/-}$ *Mice*

The genotyping procedure based on alkaline DNA extraction and TaqMan allelic discrimination proved to be reliable and was consistently applied throughout the study. All *kat2*$^{-/-}$ animals included in the breeding program and experimental cohorts carried the targeted gene deletion, which was confirmed before each experimental series. These results demonstrate that the CRISPR/Cas9-mediated deletion was stably maintained in the colony in a homozygous form, with no evidence of genetic reversion.

II. Metabolic Profiling of Tryptophan Degradation Pathways

Targeted UHPLC–MS/MS analyses revealed extensive alterations in Trp-related metabolite profiles in *kat2*$^{-/-}$ mice compared to WT controls, affecting both peripheral samples and distinct brain regions. In plasma, concentrations of KYN (**$p < 0.01$), KYNA (***$p < 0.001$), anthranilic acid (AA; **$p < 0.01$), xanthurenic acid (XA; ***$p < 0.001$), 5-hydroxyindoleacetic acid (**$p < 0.01$), and indole-3-acetic acid (IAA; ***$p < 0.001$) were reduced, whereas levels of 3-hydroxykynurenine (3-HK; **$p < 0.01$) were significantly elevated. In urine samples, a

partially divergent pattern was observed, characterized by decreased KYNA (***$p < 0.001$), XA (***$p < 0.001$), and IAA (**$p < 0.01$) levels, alongside increased concentrations of KYN (***$p < 0.001$), 3-HK (***$p < 0.001$), and 5-HT (*$p < 0.05$).

Analysis of brain tissues revealed pronounced, region-dependent metabolic remodeling. Across all examined brain regions, a uniform increase in 3-HK levels was observed (STR *$p < 0.05$; CTX *$p < 0.05$; HIPP **$p < 0.01$; CER *$p < 0.05$; STEM **$p < 0.01$). With the exception of STR, XA concentrations were reduced across regions (CTX *$p < 0.05$; HIPP *$p < 0.05$; CER *$p < 0.05$; STEM ***$p < 0.001$). Changes in KYNA were region-specific, showing decreases in the CTX and HIPP and an increase in the STR (STR *$p < 0.05$; CTX *$p < 0.05$; HIPP *$p < 0.05$). In addition, reductions were observed in Trp (CTX **$p < 0.01$; HIPP *$p < 0.05$) and quinaldic acid concentrations (CER *$p < 0.05$; STEM *$p < 0.05$). In contrast, levels of anthranilic acid (AA; CTX *$p < 0.05$) and 3-hydroxyanthranilic acid (3-HAA; CTX *$p < 0.05$) were increased in selected brain regions.

Within the 5-HT pathway, a decrease in 5-hydroxytryptophan levels (STR *$p < 0.05$; CTX ***$p < 0.001$; CER **$p < 0.01$) and region-dependent increases in 5-HT (CTX **$p < 0.01$) were observed. Along the indole-pyruvate pathway, reduced levels of IAA (HIPP **$p < 0.01$), indole-3-lactic acid (STEM ***$p < 0.001$), and indoxyl sulfate (STEM *$p < 0.05$) were detected in specific brain regions, whereas levels of indole-3-carboxaldehyde were increased (CTX ***$p < 0.001$). Within the Tyr–DA pathway, decreases were observed in Tyr (CTX *$p < 0.05$), 3,4-dihydroxyphenylacetic acid (DOPAC; CER *$p < 0.05$), biopterin (CER ***$p < 0.001$), and dihydrobiopterin (CER ***$p < 0.001$) concentrations.

Enzyme activity estimates in peripheral samples indicated reduced activities of KATs (plasma ***$p < 0.001$; urine ***$p < 0.001$), kynureninase (KYNU; AA/KYN: urine ***$p < 0.001$; 3-HAA/3-HK: plasma ***$p < 0.001$ and urine ***$p < 0.001$), monoamine oxidases (MAOs) and aldehyde dehydrogenase (ALDH) (plasma *$p < 0.05$; urine ***$p < 0.001$), as well as tryptophan-2-monooxygenase (TMO; plasma **$p < 0.01$; urine ***$p < 0.001$), together with increased activities of kynurenine-3-monooxygenase (KMO; plasma **$p < 0.01$; urine ***$p < 0.001$), tryptophan-2,3-dioxygenase and indoleamine-2,3-dioxygenase (urine ***$p < 0.001$), and aromatic L-amino acid decarboxylase (AADC; urine **$p < 0.01$). In the brain, KMO activity was increased across all regions (STR **$p < 0.01$; CTX **$p < 0.01$; HIPP ***$p < 0.001$; CER ***$p < 0.001$; STEM ***$p < 0.001$). In addition, while activities of KAT (STR *$p < 0.05$), AADC (STR *$p < 0.05$; CTX **$p < 0.01$; CER **$p < 0.01$), and catechol-O-methyltransferase (CER *$p < 0.05$) were increased in certain brain regions, activities of KYNU (3-HAA/3-HK: HIPP *$p < 0.05$), kynurenine aminotransferase III (CTX **$p < 0.01$; HIPP **$p < 0.01$; CER

**p < 0.01; STEM ***p < 0.001), tryptophan hydroxylases (CTX **p < 0.01; CER **p < 0.01), MAOs and ALDH (CTX *p < 0.05), and MAOs involved in DOPAC conversion within the DA pathway (CER *p < 0.05) were reduced. TMO activity did not show a uniform direction of change across brain regions: it was decreased in the HIPP (*p < 0.05), while increased activity was observed in the CER (*p < 0.05).

Both OSI and EI were significantly elevated in peripheral samples of *kat2*$^{-/-}$ mice (OSI: plasma ***p < 0.001; urine ***p < 0.001; EI: plasma ***p < 0.001; urine ***p < 0.001). At the central nervous system level, OSI was increased in multiple brain regions (CTX **p < 0.01; HIPP **p < 0.01; CER **p < 0.01; STEM *p < 0.05), whereas the elevation of excitotoxicity was primarily confined to the HIPP (*p < 0.05).

III. Baseline Phenotypic Characterization of kat2$^{-/-}$ Mice

Baseline phenotypic assessment using the modified SHIRPA protocol revealed no significant differences between *kat2*$^{-/-}$ mice and WT control animals across any of the evaluated parameters.

IV. Behavioral Characterization of kat2$^{-/-}$ Mice

In the OFT, *kat2*$^{-/-}$ mice exhibited reduced locomotor activity, as indicated by a decrease in total ambulation distance (**p < 0.01). In addition, they performed fewer jumps (*p < 0.05) and displayed attenuated exploratory behavior, reflected by a lower number of entries into the central and corner zones (central zones: *p < 0.05; corner zones: ***p < 0.001). In contrast, no genotype-dependent differences were detected in the rotarod test, indicating preserved motor coordination and balance. No significant differences between *kat2*$^{-/-}$ and WT mice were observed in anxiety-like behaviors assessed using the EPM, LDB, or MBT. In the FST used to assess depression-like behavior, *kat2*$^{-/-}$ mice showed increased immobility time (*p < 0.05) and reduced swimming activity (*p < 0.05). However, no genotype-dependent differences were detected in TST. Assessment of cognitive and attentional functions revealed no significant genotype-dependent differences in recognition memory (NORT), aversive learning (PAT), attentional performance (OBAT), or working memory (Y-maze test). During the 3CT, both groups exhibited comparable behavior, and no genotype-dependent differences were detected across any of the evaluated social parameters.

Discussion

Genetic validation and model integrity

Genetic stability of the experimental model is necessary for accurate interpretation of metabolic and behavioral results. In the present study, genotyping confirmed that deletion of the *aadat*

gene was consistently present in all *kat2*$^{-/-}$ animals. The absence of heterozygous genotypes, together with segregation of *kat2*$^{-/-}$ and WT alleles, indicates that the CRISPR/Cas9-mediated gene knockout was stably inherited in a homozygous form, with no evidence of allelic reversion or mosaicism. The high specificity and reproducibility of the genotyping strategy prevented the inclusion of animals with partial or ambiguous genotypes, thereby minimizing variability arising from genetic background. Accordingly, the observed metabolic remodeling and functional alterations can be attributed to the consequences of KAT II deficiency. Confirmation of the model's genetic integrity thus provides a solid foundation for interpreting how targeted disruption of a single key enzyme propagates across interconnected Trp metabolic pathways.

Metabolic remodeling and pathway dominance following KAT II deletion

Genetic deletion of *aadat* induced extensive yet spatially heterogeneous remodeling of Trp metabolism across peripheral and central compartments. In plasma and urine, the metabolic profile consistently shifted toward elevated 3-HK, accompanied by reduced KYNA and XA levels, indicating attenuation of antioxidant branches of the KYN pathway and dominance of pro-oxidant directions. These peripheral alterations were paralleled by changes in serotonergic and indole-derived metabolites, suggesting that KAT II deficiency affects multiple interconnected Trp-derived pathways. In the brain, metabolic remodeling displayed pronounced regional specificity. The uniform elevation of 3-HK together with a global reduction in XA points to a generalized oxidative bias, whereas region-dependent alterations in KYNA—decreases in the CTX and HIPP and an increase in the STR—suggest activation of local compensatory mechanisms. Analysis of enzyme activity ratios further confirmed the dominance of the KMO-driven oxidative branch, while KAT-mediated protective routes were constrained in several regions. Importantly, these findings indicate that targeted disruption of KAT II does not remain confined to the KYN pathway. Parallel alterations observed in the 5-HT, indole-pyruvate, and Tyr–DA systems support the concept that Trp metabolism operates as an integrated network, in which perturbation of a single enzymatic node induces systems-level metabolic reorganization. Accordingly, KAT II deficiency should be interpreted not as a pathway-specific alteration, but as a network-level disturbance of metabolic balance.

Latent oxidative and excitotoxic vulnerability

KAT II deficiency establishes a metabolic environment characterized by increased oxidative load and context-dependent excitotoxic vulnerability across both peripheral and central compartments. Composite indices derived from KYN metabolites consistently indicated a pro-oxidant shift in *kat2*$^{-/-}$ mice. Elevation of OSI was observed not only in multiple brain regions

but also in plasma and urine, suggesting that disruption of redox balance represents a systemic consequence of reduced KYNA availability. EI was likewise increased in peripheral samples, whereas within the brain, it exhibited pronounced regional specificity, with a significant increase detected exclusively in the HIPP. This dissociation indicates that while peripheral indices reflect a generalized state of vulnerability, central excitotoxic risk is governed by local regulatory and buffering mechanisms. Importantly, the concurrent elevation of oxidative stress and excitotoxicity did not result in overt neurotoxicity or baseline behavioral impairment. Instead, KAT II deficiency defines a latent vulnerability state that lowers tolerance to functional imbalance without inducing pathology on its own. Such a state may render stress-sensitive brain regions, most notably the HIPP, more susceptible to secondary challenges, including stress, aging, or inflammatory burden.

Behavioral resilience despite neurochemical imbalance

Despite the pronounced and region-dependent neurochemical remodeling observed across Trp metabolic pathways, the baseline phenotype and behavioral function of *kat2*$^{-/-}$ mice remained largely preserved. Screening using the modified SHIRPA protocol revealed no genotype-dependent differences in basic neurological, autonomic, or sensorimotor functions, indicating effective compensatory mechanisms in young adulthood. Behavioral alterations were primarily confined to domains related to spontaneous exploration and stress adaptation, while anxiety-like behavior, cognitive performance, social interaction, and aversive memory remained intact. In the OFT, reduced exploratory activity was observed in the absence of motor impairment, whereas the modified FST revealed increased immobility and reduced swimming time. In contrast, no alterations were detected in the tail suspension test, highlighting functional differences between stress-related paradigms. Collectively, these findings suggest that KAT II deficiency establishes a latent, stress-sensitive behavioral state in which neurochemical imbalance preferentially affects coping-related behaviors without resulting in generalized behavioral impairment. This phenotype may serve as a preclinical model for investigating mechanisms of neuropsychiatric vulnerability and resilience.

Limitations and future directions

Several limitations should be considered when interpreting the present findings. The experiments were conducted under baseline conditions in young adult male mice, which limits conclusions regarding developmental trajectories, aging-related effects, and sex-dependent differences. Given the known sexual dimorphism in Trp metabolism and stress responsivity, inclusion of female cohorts and longitudinal designs will be essential to elucidate the long-term consequences of KAT II deficiency. Metabolomic analyses were based on regional tissue

homogenates, which may obscure cell-type-specific alterations. Future studies employing cell-resolved or spatially resolved approaches will be necessary to localize metabolic remodeling precisely. Enzyme activities were inferred from product-to-substrate ratios, providing system-level insights but not replacing direct kinetic measurements. In addition, the gut microbiome was not directly characterized, limiting the interpretation of microbial contributions. Finally, behavioral testing did not include targeted stress or cognitive challenges. Future paradigms designed to probe such conditions will be required to reveal the functional consequences of the latent metabolic vulnerability identified in this study.

Conclusions

The present work provides a comprehensive characterization of the neurochemical and behavioral consequences of genetic deletion of the *aadat* gene encoding KAT II in young adult *kat2*$^{-/-}$ mice, integrating region-specific metabolomic analyses, inferred enzyme activities, OSI and EI, and behavioral assessments. Our results demonstrate that KAT II deficiency induces pronounced yet spatially heterogeneous remodeling of Trp metabolism across both peripheral and central compartments, while behavioral alterations remain selective, modest, and context dependent. At the biochemical level, *aadat* deletion shifted Trp degradation toward a pro-oxidant and potentially excitotoxic profile, characterized by global elevation of 3-HK, reduced XA, region-specific modulation of KYNA, and reorganization of serotonergic, indole-pyruvate, and Tyr–DA pathways. These changes were accompanied by increased OSI and EI, particularly within cortico-hippocampal regions, indicating latent metabolic vulnerability rather than overt neurodegeneration. Concordant alterations observed in peripheral samples support the translational relevance of peripheral biomarkers as indicators of central pathway imbalance. Despite this marked neurochemical disequilibrium, behavioral performance across most cognitive, social, and anxiety-related domains remained preserved, highlighting the compensatory capacity of the central nervous system under baseline conditions. Nevertheless, selective differences emerged in paradigms assessing spontaneous activity and stress-related behavioral responses, primarily as reduced exploratory activity and increased immobility. Overall, these findings support the concept that KAT II deficiency does not produce constitutive psychopathology but rather establishes a state of metabolic and affective vulnerability. Refinement of the role of KAT II within the KYN pathway and interconnected Trp metabolic routes provides a mechanistic framework that may contribute to understanding neuropsychiatric vulnerability and inform future development of targeted, metabolism-based therapeutic strategies.

Acknowledgement

I would like to express my sincere gratitude to my supervisors, Professor László Vécsei and Dr. Masaru Tanaka, for their continuous guidance, professional advice, and support throughout my doctoral studies. I am especially thankful for their role in shaping my scientific thinking and for their assistance in my academic and scientometric development.

I also thank Professor Péter Klivényi, Head of Department, for providing the opportunity to conduct my doctoral work and related research at the Department of Neurology.

I am grateful to all current and former members of the HUN-REN–SZTE Neuroscience Research Group, with special thanks to Dr. Diána Martos, Dr. Zsuzsanna Fülöpné Bohár, Dr. Annamária Fejes-Szabó, and Dr. Eleonóra Spekker, whose support and mentorship greatly contributed to my scientific progress. I would also like to thank our laboratory assistant, Erzsébet Lukács, for her invaluable help with the technical aspects of laboratory work.

I extend my appreciation to our collaboration partners, Dr. Zsolt Galla, Dr. Péter Monostori, Dr. László Juhász, Krisztina Spisák, Dr. Mónika Szűcs, Professor Mihály Boros, and Professor József Toldi, whose professional input and fruitful cooperation significantly advanced our research. Additionally, I am deeply thankful to Professor Etsuro Ono and his colleagues at Kyushu University, Fukuoka, for providing the genetically modified mouse strains essential for this work.

Finally, I would like to express my deepest gratitude to my beloved family and friends for their unconditional love, patience, continuous support, and for standing by me and believing in me throughout these challenging years. *"**Sub pondere crescit palma.**"*

This work was financially supported by the GINOP-2.3.2-15-2016-00034 grant, the National Research, Development, and Innovation Office–NKFIH (K138125), the SZTE SZAOK-KKA program (No. 2022/5S729), the HUN-REN Hungarian Research Network, the Japan Society for the Promotion of Science (JSPS) through its Joint Research Projects under Bilateral Programs (Grant No. JPJSBP120203803), and the Hungarian Academy of Sciences (NKM-65/1/2021). During my doctoral studies, I received partial support from the Doctoral School of Clinical Medicine, Albert Szent-Györgyi Medical School, University of Szeged.

During the preparation of my thesis, I used Grammarly and ChatGPT 5.2 for grammar checking, correction, and refinement of scientific language.