medical sciences forum

MDPI

Kynurenine Aminotransferase II Knockout Mice Mimic Depression with Psychomotor Retardation

Ágnes Szabó [1,2,†], Masaru Tanaka [3,†,‡], Eleonóra Spekker [3], József Toldi [4], Etsuro Ono [5,6] and László Vécsei [1,3,‡]

[1] Department of Neurology, Albert Szent-Györgyi Medical School, University of Szeged, Semmelweis u. 6, H-6725 Szeged, Hungary
[2] Doctoral School of Clinical Medicine, University of Szeged, Korányi fasor 6, H-6720 Szeged, Hungary
[3] Danube Neuroscience Research Laboratory, ELKH-SZTE Neuroscience Research Group, Eötvös Loránd Research Network, University of Szeged (ELKH-SZTE), Tisza Lajos krt. 113, H-6725 Szeged, Hungary
[4] Department of Physiology, Anatomy and Neuroscience, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary
[5] Department of Biomedicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[6] Center of Biomedical Research, Research Center for Human Disease Modeling, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
* Correspondence: Correspondence: Correspondence: vecsei.laszlo@med.u-szeged.hu (L.V.), tanaka.masaru.1@med.u-szeged.hu (M.T.); Tel.: +36-62-545-351 (L.V.; M.T.)
These authors contributed equally to this work.

Abstract: Major depressive disorder (MDD) exhibits negative emotional symptoms with various degrees of psychomotor manifestation including anxiety and motor impairment. Alteration of tryptophan-kynurenine (KYN) metabolism has been implicated in the pathogenesis of MDD. Kynurenine/α-aminoadipate aminotransferase (KAT II) is a mitochondrial enzyme that catalyzes the reaction of KYN to kynurenic acid. Little is known about the impact of the gene *aadat* knockout (*aadat*$^{-/-}$ *aka kat2*$^{-/-}$) on behaviors. We studied negative emotional and motor domains at 4 and 12 weeks of male *kat2*$^{-/-}$ mice and the wild-type counterpart C57BL/6N. Depression-like and anxiety-like behaviors and motor functions were assessed by modified forced swim test (FST), light-dark box (LDB) test, and open field (OF) test. Modified FST showed significantly higher depression-like behaviors at 4 and 12 weeks in *kat2*$^{-/-}$ mice than the wild-type and in an age-dependent manner. In the LDB test both strains spent significantly more time in the light at week 12 than at week 4. In the OF test both strains entered significantly more time to the center zone at week 12 than at week 4 and showed significantly longer ambulation distance at week 12. We show here that *kat2*$^{-/-}$ mice exhibit increasing depression-like behavior, accompanied with decreasing anxiety and motor activity in an age-dependent manner. Thus, the *aadat* gene knockout influences emotional behavioral domains in such a manner that the strain potentially serves as an animal model of MDD subtype, depression with psychomotor retardation.

Keywords: depression; anxiety; motor function; kynurenine; kynurenine aminotransferase; psychomotor; animal model; forced swim test; open field test; light-dark box test

Citation: Neurology, D.o.; School, A.S.-G.M.; Szeged, U.o.; Semmelweis u.; H-6725 Szeged; Hungary Ágnes Szabó [1,2,†], Masaru Tanaka [3,†,‡], Eleonóra Spekker [3], József Toldi [4], Etsuro Ono [5,6] and László Vécsei [1,3,*] **2023**, *2*, x.

https://doi.org/10.3390/xxxxx

Published: 24 March 2023

1. Introduction

Major depressive disorder (MDD) is a common and serious emotional mental illness characterized by pervasive low mood, loss of pleasure, feelings of guilt, and lack of energy; in addition, it typically exhibits multifarious manifestation including anxiety and motor impairment, presenting various symptom clusters [1]. The tryptophan (Trp)-kynurenine (KYN) metabolic pathway is a major route of Trp catabolism, producing several bioactive molecules [2]. Trp-KYN metabolism is found to be altered in patients with MDD [3]. However, little is known about the roles of altered Trp-KYN metabolism in the pathogenesis of MDD [4]. Kynurenine/α-aminoadipate aminotransferase (KAT II) encoded by the *aadat* gene is a mitochondrial enzyme of the Trp-KYN metabolic system, which catalyzes the reaction of KYN to kynurenic acid (Figure 1) [5]. No human disease

has been associated with the *aadat* gene variants [6]. Kynurenic acid is a receptor antagonist of glutamate receptors and is considered to possess beneficial effects. However, the action may be limited to a certain concentration and microenvironment [7,8]. A few studies reported the behaviors of the *aadat* gene knockout (*kat2*$^{-/-}$) mice, but the consequence of the gene deletion remains inconclusive [9,10].

We assayed the negative emotional and motor domains of behavior in male *kat2*$^{-/-}$ mice by animal models of depression, anxiety, and motor impairment to reveal the impact of the *aadat* gene deletion on behaviors.

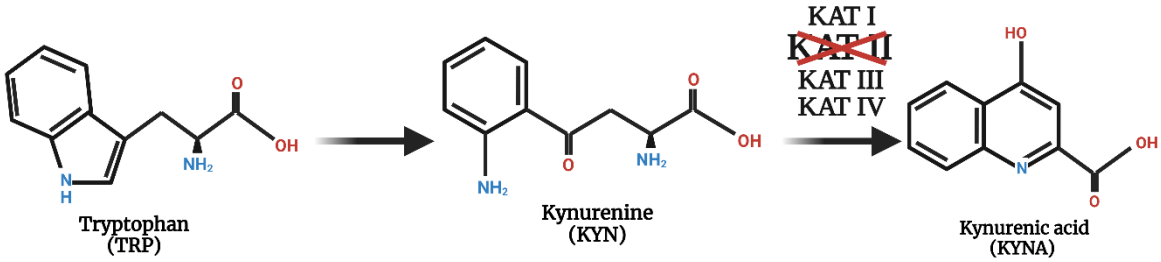

Figure 1. Synthesis of kynurenic acid and the participation of kynurenine aminotransferase isoforms.

2. Materials and Methods

Behavioral sampling was carried out with modified forced swim test (FST), light-dark box (LDB) test, and open field (OF) test at 4 and 12 weeks of age in male *kat2*$^{-/-}$ mice and wild type (WT) counterpart, C57BL/6N (n = 10-14).

In accordance with the guidelines of the 8th Edition of the Guide for the Care and Use of Laboratory Animals and the Use of Animals in Research of the International Association for the Study of Pain and the directive of the European Economic Community (86/609/ECC). The experiments were approved by the Committee of Animal Research at the University of Szeged (I-74-1/2022) and the Scientific Ethics Committee for Animal Research of the Protection of Animals Advisory Board (XI./95/2020).

2.1. Modified forced swim test (FST)

The modified FST was performed as reported previously [11]. The mice were placed individually in a glass cylinder of 12 cm in diameter and 30 cm in height. Water (25±1 °C) was filled to a height of 20 cm. Fresh water was used for each mouse. A 15-min pretest was carried out 24 h before the 3-min test session. A time-sampling technique was conducted to count the duration of climbing, swimming, and immobility times.

2.2. Light-dark box (LDB) test

The LDB apparatus consists of larger illuminated (2/3 of the box) and smaller dark compartments (1/3 of the box), connected by a 5x5 cm door. The duration of time spent in the lighted compartment in the 5-minutes session was measured 5 seconds after a mouse was placed in the bright part [12]. The box was wiped after each session with 70% ethanol and aired for 5 minutes.

2.3. Open field (OF) test

The OF box measuring length of 48 cm and height of 40 cm was illuminated in the center of the box by an ordinary table lamp and the Conducta 1.0 system (Experimetria Ltd.) tracked the movement of the mouse. Each mouse was placed in the center of the OF box. Ambulation distance, time spent in the center zone and number of entries to the center zone was measured for 10 minutes [12]. The box was wiped after each session with 70% ethanol and aired for 5 minutes [13].

2.4. Statistical analysis

The Shapiro–Wilk test and the Q-Q plot were applied to determine the distribution of data and to confirm the distribution, respectively. One-way ANOVA test evaluates the values of the behavioral tests conducted, followed by the Tamhane post hoc test.

3. Results

Modified FST showed that the immobility time was significantly longer in *kat2*$^{-/-}$ mice than that of WT in both age groups and the immobility time of 12-week mice was significantly longer compared to that of 4-week mice in both strains. The climbing time was significantly shorter in *kat2*$^{-/-}$ mice than that of WT at 4 weeks and the climbing time of 12-week mice was significantly shorter compared to that of 4-week mice in both strains. The swimming time was significantly shorter in *kat2*$^{-/-}$ mice than that of WT at 12 weeks and it was significantly shorter in 12-weeks mice than that of 4-week mice in both strains (Figure 2, Table 1).

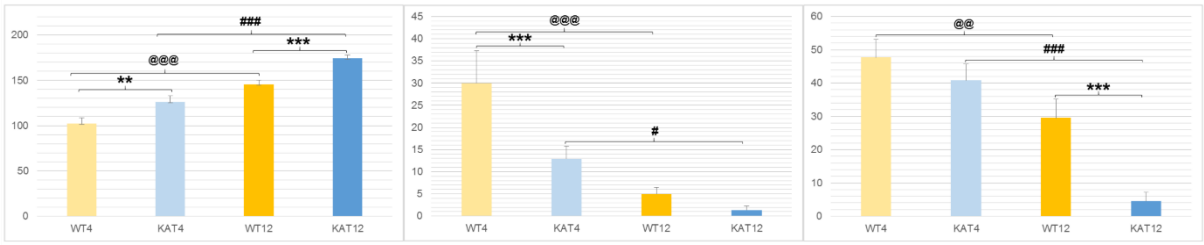

Figure 2. Time spent with immobility (s; left), climbing (s; middle) and swimming (s; right) in modified forced swim test. WT4: 4-weeks old wild-type; KAT4: 4-weeks old *kat2*$^{-/-}$ mice; WT12: 12-weeks old wild-type; KAT12: 12-weeks old *kat2*$^{-/-}$ mice; *: significant differences between wild type and *kat2*$^{-/-}$ strains in the same age group; @: significant differences between wild type age groups; #: significant differences between age groups of *kat2*$^{-/-}$ mice; mean+SEM; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table 1. Statistical values of behavioral tests. Tests, number of animals, average, SEM, p value.

LDB test showed that both strains spent significantly more time in the lighted chamber at week 12 than at week 4, but no significant difference was observed between the strains in both age groups (Figure 3, Table 1).

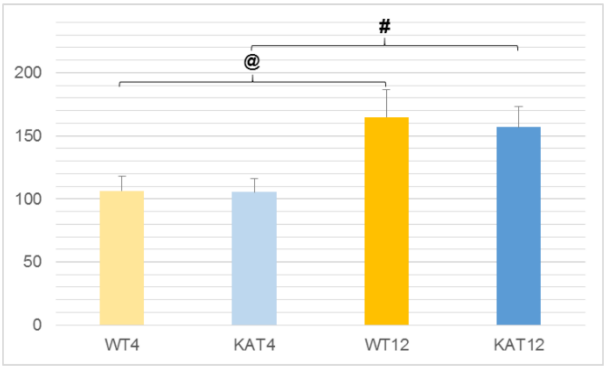

Figure 3. Time spent in the lighted compartment at week 4 and week 12 in light-dark box test. WT4: 4-weeks old wild-type; KAT4: 4-weeks old *kat2*$^{-/-}$ mice; WT12: 12-weeks old wild-type; KAT12: 12-weeks old *kat2*$^{-/-}$ mice; *: significant differences between wild type and *kat2*$^{-/-}$ strains in the same age

group; @: significant differences between wild type age groups; #: significant differences between age groups of *kat2*$^{-/-}$ mice; mean+SEM; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

The OF test likewise showed that both strains entered more times into the lighted center area at 12 week than at week 4, but no significant difference was observed between the strains in both age groups. The ambulation distance was significantly longer at week 12 than at week 4, and it was significantly shorter in *kat2*$^{-/-}$ mice than that of WT at 12 week (Figure 4, Table 1).

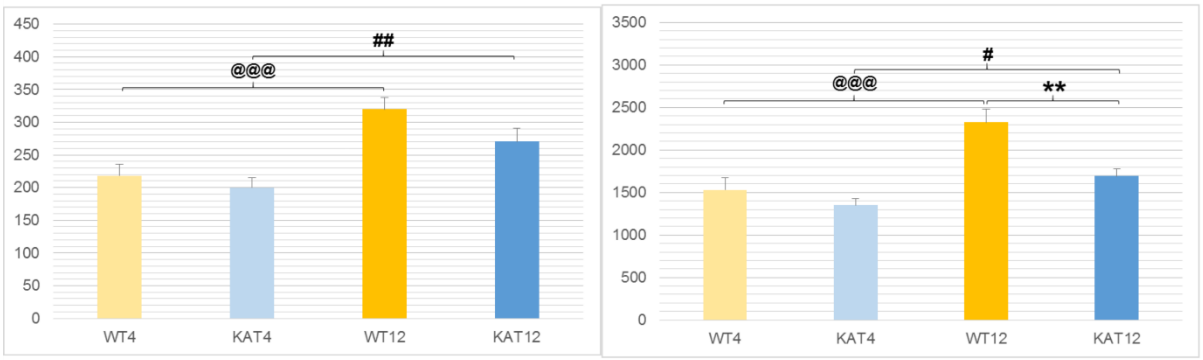

Figure 4. Number of entries to the lighted center zones (s; left) and ambulation distance (cm; right) in open field test. WT4: 4-weeks old wild-type; KAT4: 4-weeks old *kat2*$^{-/-}$ mice; WT12: 12-weeks old wild-type; KAT12: 12-weeks old *kat2*$^{-/-}$ mice; *: significant differences between wild type and *kat2*$^{-/-}$ strains in the same age group; @: significant differences between wild type age groups; #: significant differences between age groups of *kat2*$^{-/-}$ mice; mean+SEM; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

The *kat2*$^{-/-}$ mice revealed significantly increasing depression-like behaviors in an age-dependent manner, while both strains showed decreasing anxiety-like behavior with age and *kat2*$^{-/-}$ mice elicited significantly decreased motor activity at 12 weeks.

4. Discussion

We studied the depression-like and anxiety-like behaviors and motor function of male *kat2*$^{-/-}$ mice by modified FST, LDB test, and OF test, to investigate the impact of the *aadat* gene deletion on behaviors.

Our study revealed that *kat2*$^{-/-}$ mice exhibits an increasing depression-like behavior in an age-dependent manner, with significantly decreasing climbing time at 4 week and significantly decreasing swimming time at 12 week (Figure 2, Table 1). Both *kat2*$^{-/-}$ mice and wild type counterpart spent significantly more time in the bright chamber of LDB and significantly more often entered into the lighted center of OF at week 12 than at week 4 (Figures 3,4, Table 1). Furthermore, the ambulation distance was significantly shorter in *kat2*$^{-/-}$ mice than that of WT at 12 week (Figure 4, Table 1).

The previous studies showed that transitory hyperlocomotive activity and abnormal motor coordination during postnatal day 17 to 26, which returned to normal levels afterwards and increased cognitive functions at week 3 [9,10].

This study shows the presence of deterioration in depression and motor function in a growing stage of *kat2*$^{-/-}$ mice and characteristic improvement in anxiety in a growing stage of both strains. The authors acknowledge that this study is to extend to other behavioral domains closely associated with depression. An animal model of the positive valence of emotional domain such as sucrose preference test may help further characterize the phenotype of *kat2*$^{-/-}$ mice. Furthermore, neurochemical basis of changes in emotional and motor functions is to be explored. First of all, measuring Trp metabolites including serotonin, 5-hydroxyindole acetic acid, and KYNs may clarify the impact of the gene *kat2* knockout on Trp metabolism. Probing brain-derived neurotrophic factor and c-fos in the

pertinent regions of the brain may help elucidate the neuronal basis of age-dependent behavioral change in the growing stage.

5. Conclusions

We show that *kat2*$^{-/-}$ mice exhibit increasing depression-like behavior, accompanied with decreasing anxiety and motor activity in an age-dependent manner. Thus, the *aadat* gene knockout influences behavioral domains in such a manner that the transgenic strain potentially serves as an animal model of MDD subtype, depression with psychomotor retardation.

Author Contributions: conceptualization, M.T. and L.V.; methodology, Á.S. and M.T.; software, Á.S. and E.S.; validation, Á.S.,M.T., and E.S; formal analysis, E.S.; investigation, Á.S. and M.T.; resources, J.T. and E.O.; data curation, Á.S., M.T. and E.S.; writing—original draft preparation, Á.S. and M.T.; writing—review and editing, Á.S., M.T. and L.V.; visualization, Á.S.; supervision, L.V.; project administration, J.T., E.O., and L.V.; funding acquisition, J.T., E.O., and L.V.

Funding: This research was funded by National Research, Development and Innovation Office – NKFIH 138125, MTA-JSP-050609, and ELKH-SZTE.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maes, M. "Functional" or "psychosomatic" symptoms, e.g. a flu-like malaise, aches and pain and fatigue, are major features of major and in particular of melancholic depression. *Neuro. Endocrinol. Lett.* **2009**, *30(5)*, 564-573.
2. Tanaka, M.; Bohár, Z.; Vécsei, L. Are Kynurenines Accomplices or Principal Villains in Dementia? Maintenance of Kynurenine Metabolism. *Molecules* **2020**, *25*, 564. https://doi.org/10.3390/molecules25030564
3. 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. https://doi.org/10.3390/biomedicines9070734
4. Marx, W.; McGuinness, A.J.; Rocks, T.; Ruusunen, A.; Cleminson, J.; Walker, A.J.; Gomes-da-Costa, S.; Lane, M.; Sanches, M.; Diaz, A.P.; et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: A meta-analysis of 101 studies. *Mol. Psychiatry* **2021**, *26*, 4158–4178.
5. Tanaka, M.; Toldi, J.; Vécsei, L. Exploring the Etiological Links behind Neurodegenerative Diseases: Inflammatory Cytokines and Bioactive Kynurenines. *Int. J. Mol. Sci.* **2020**, *21*, 2431.https://doi.org/10.3390/ijms21072431
6. UniProtKB—Q8N5Z0 AADAT_HUMAN. Available online: https://www.uniprot.org/uniprotkb/Q8N5Z0/entry (accessed on on 26 February 2023).
7. Tanaka, M.; Bohar, Z.; Martos, D.; Telegdy, G.; Vecsei, L. Antidepressant-like effects of kynurenic acid in a modified forced swim test. *Pharmacol. Rep.* **2020**, *72*, 449–455. https://doi.org/10.1007/s43440-020-00067-5
8. Martos, D.; Tuka, B.; Tanaka, M.; Vécsei, L.; Telegdy, G. Memory Enhancement with Kynurenic Acid and Its Mechanisms in Neurotransmission. *Biomedicines* **2022**, *10*, 849. https://doi.org/10.3390/biomedicines10040849
9. Yu, P.; Di Prospero, N.A.; Sapko, M.T.; Cai, T.; Chen, A.; Melendez-Ferro, M.; Du, F.; Whetsell, W.O., Jr.; Guidetti, P.; Schwarcz, R.; et al. Biochemical and phenotypic abnormalities in kynurenine aminotransferase II-deficient mice. *Mol. Cell. Biol.* **2004**, *24*, 6919–6930. https://doi.org/10.1128/MCB.24.16.6919-6930.2004.
10. Potter, M.C.; Elmer, G.I.; Bergeron, R.; Albuquerque, E.X.; Guidetti, P.; Wu, H.Q.; Schwarcz, R. Reduction of endogenous kynurenic acid formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior. *Neuropsychopharmacology* **2010**, *35*, 1734–1742. https://doi.org/10.1038/npp.2010.39.
11. Khisti, R.T.; Chopde, C.T.; Jain, S.P. Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. *Pharmacol. Biochem. Behav.* **2000**, *67(1)*, 137–143. https://doi.org/10.1016/s0091-3057(00)00300-2
12. Szalardy, L.; Molnar, M.F.; Zadori, D. Cseh. E.K., Veres, G.; Kovacs, G.G.; Vecsei, L.; Klivenyi, P. Non-motor Behavioral Alterations of PGC-1α-Deficient Mice - A Peculiar Phenotype With Slight Male Preponderance and No Apparent Progression. *Front. Behav. Neurosci.* **2018**, *12*, 180. https://doi.org/10.3389/fnbeh.2018.00180
13. Giménez-Llort, L.; Marin-Pardo, D.; Marazuela, P.; Hernández-Guillamón, M. Survival Bias and Crosstalk between Chronological and Behavioral Age: Age- and Genotype-Sensitivity Tests Define Behavioral Signatures in Middle-Aged, Old, and Long-Lived Mice with Normal and AD-Associated Aging. *Biomedicines* **2021**, *9*, 636. https://doi.org/10.3390/biomedicines9060636