NBRP Rat & Mouse News

Functional analysis tools for GAD isoforms

Vol.1 September 2022

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system. GABA is synthesized from glutamate by glutamate decarboxylase (GAD). GAD exists in two isoforms with different molecular weights, GAD67 and GAD65, which are independently encoded by the *Gad1* and *Gad2* genes, respectively. GAD65 and GAD67 are highly homologous with 65% sequence identity, but are distinguished by molecular weights, cofactor interactions, and subcellular distribution. GAD65 and GAD67 are coexpressed in GABAergic neurons. GAD65 synthesizes and releases GABA in an activity-dependent manner, whereas GAD67 maintains basal GABA levels. Since these two GAD isoforms have been reported to be associated with a number of human neuropsychiatric disorders, there are high expectations for their functional analysis.

Rat resources

Gad1 and *Gad2* KO strains of rats have been established using the CRISPR/Cas9 or TALEN technology. The *Gad1*/*Gad2* double KO rats, generated by crossing double hetero KO rats of each gene, do not have GABA in their brain as in mice, and die at birth. However, *Gad1* and *Gad2* KO show phenotypic differences between rats and mice (Table 1). The brain GABA content in *Gad1* KO mice is reduced to 7% of wild-type, whereas 18% of wild-type is synthesized in *Gad1* KO rats, suggesting that the contribution of both isoforms in GABA synthesis may be different between rats and mice (the brain GABA content in *Gad2* KO mice and rats is 87% and 71% of that in wild-type, respectively). Moreover, *Gad1* KO rats can survive till adulthood, allowing phenotypic analysis. Rats have the advantage of being approximately 10 times larger than mice and can be used for complex behavioral analysis. *Gad1* and *Gad2* KO rats are deposited in the National BioResource Project - Rat, and they are available.

Depositor	Yuchio Yanagawa, M.D., Ph.D (Gunma University)
Strain name	・LE-Gad1<em15Yyan> (Gad1 KO rats) (NBRP Rat No. 0906) ・LE-Gad2<em24Yyan> (Gad2 KO rats) (NBRP Rat No. 0907)
References	・CRISPR/Cas9-engineered Gad1 elimination in rats leads to complex behavioral changes: implications for schizophrenia. Fujihara K, Yamada K, Ichitani Y, Kakizaki T, Jiang W, Miyata S, Suto T, Kato D, Saito S, Watanabe M, Kajita Y, Ohshiro T, Mushiake H, Miyasaka Y, Mashimo T, Yasuda H, Yanagawa Y. Transl Psychiatry. 2020 Dec 8;10(1):426. ・Rats deficient in the GAD65 isoform exhibit epilepsy and premature lethality. Kakizaki T, Ohshiro T, Itakura M, Konno K, Watanabe M, Mushiake H, Yanagawa Y. FASEB J. 2021 Feb;35(2):e21224. ・Impact of GAD65 and/or GAD67 deficiency on perinatal development in rats. Jiang W, Kakizaki T, Fujihara K, Miyata S, Zhang Y, Suto T, Kato D, Saito S, Shibasaki K, Ishizaki Y, Isoda K, Yokoo H, Obinata H, Hirano T, Miyasaka Y, Mashimo T, Yanagawa Y. FASEB J. 2022 Feb;36(2):e22123.

Table 1. Characteristics of GAD isoforms

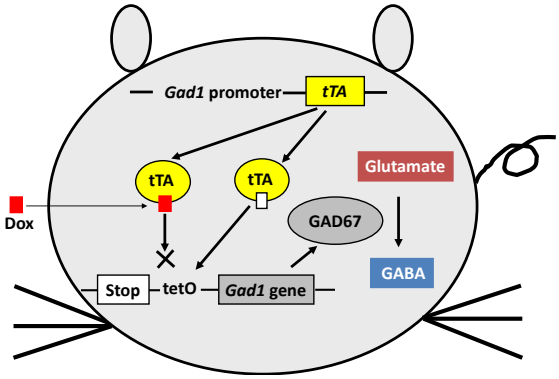
	GAD65	GAD67
Gene	Gad2	Gad1
Molecular weight	65,000 Da	67,000 Da
Amino acids	585	593
Phenotypes of knockout mouse	Increased sensitivity to pain Epileptic seizure Any Gad2 KO mice did not die until postnatal week 4 (P4W), but 25% of them die until P25W.	Cleft palate, Omphalocele All Gad1 KO mice die at birth date (P0D).
Phenotypes of knockout rat	Epileptic seizure 80% of Gad2 KO rats died until P4W.	Lower body weight Impairments in spatial learning and memory 33% of Gad1 KO rats survived to adulthood.

(Note) Molecular weights and the number of amino acids are similar in GAD proteins between mice and rats.

Courtesy of Yuchio Yanagawa, M.D., Ph.D.

Mouse resources

Gad1 KO mice are known to be neonatal lethal. Therefore, neurobiological consequences of GAD67 dysfunction in the mature brain are poorly understood. As a tool to analyze effects of global loss of GAD67 in adults, RIKEN BRC can provide GAD67 knockdown mice that utilize a tetracycline-regulated gene expression suppression system (Tet-off system) (Fig. 1). Briefly, GAD67 knockdown mice can be produced by crossing two strains. One is a Gad1-Stop-tetO KI strain (RBRC11364) in which a STOP sequence followed by a tetO sequence is inserted upstream of *Gad1* translation start site. The other is a Gad1-tTA KI strain (RBRC11365) which expresses tTA under control of endogenous *Gad1* promoter. The double KI mice can survive to adulthood and suppress GAD67 protein expression upon doxycycline administration. In fact, GAD67 knockdown mice have been reported to show behavioral abnormalities. Therefore, further analysis is expected to contribute to neuropsychiatric disease-related research.



Courtesy of Yuchio Yanagawa, M.D., Ph.D.

Fig. 1. GAD67 knockdown mice using the Tet-off system.
Before doxycycline (Dox) treatment, tTA binds to the tetO site and promotes *Gad1* transcription and GAD67 production. Dox administration interferes with tTA binding to tetO site and suppresses *Gad1* transcription and GAD67 production.

Depositor	Yuchio Yanagawa, M.D., Ph.D (Gunma University)
Strain name	・B6-Gad1<tm1(tetO)Ksak> (Gad1-Stop-tetO KI mice) RBRC11364 ・B6;Cg-Gad1<tm1.1(tTA2)Kftnk> (Gad1-tTA KI mice) RBRC11365
References	・Global knockdown of glutamate decarboxylase 67 elicits emotional abnormality in mice. Miyata S, Kakizaki T, Fujihara K, Obinata H, Hirano T, Nakai J, Tanaka M, Itohara S, Watanabe M, Tanaka KF, Abe M, Sakimura K, Yanagawa Y. Mol Brain. 2021 Jan 7;14(1):5. ・Expanding the repertoire of optogenetically targeted cells with an enhanced gene expression system Tanaka KF, Matsui K, Sasaki T, Sano H, Sugio S, Fan K, Hen R, Nakai J, Yanagawa Y, Hasuwa H, Okabe M, Deisseroth K, Ikenaka K, Yamanaka A. Cell Rep. 2012 Aug 30;2(2):397-406.