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LETTER

The de novo *GABRA4* p.Thr300Ile variant found in a patient with early-onset intractable epilepsy and neurodevelopmental abnormalities displays gain-of-function traits

To the Editors:

We were encouraged to read the recent publication by Vogel et al.[1] in *Epilepsia* providing the first association between a de novo missense variant in *GABRA4* and a neurodevelopmental disorder with early-onset epilepsy.[1]

The *GABRA4* gene encodes the α4 subunit of the γ-aminobutyric acid (GABA) type A receptor, and this subunit is relatively abundant in the cortex, hippocampus, and thalamus, all brain regions known to be involved in epilepsy.[2,3] The α4 subunit primarily assembles with β and δ subunits to form, for example, α4β2δ receptors,[4–6] and these receptors are localized in extrasynaptic membranes where they respond to low ambient levels of GABA and spill over from synaptic release resulting in long-lasting tonic inhibition of neuronal activity.[7–9]

We recently discovered that pathogenic variants in *GABRD*, encoding the δ subunit, cause gain-of-function traits in α4β2δ receptors and interestingly one specific variant, *GABRD* p.Thr291Ile, is paralogous to the *GABRA4* p.Thr300Ile variant.[10] Intrigued by this observation, we extended the study of Vogel et al.[1] with electrophysiological analysis of the *GABRA4* p.Thr300Ile variant in combination with the δ subunit using previously described methodologies.[10,11]

The mean current amplitude obtained with a maximally efficacious concentration of GABA was increased by 6.2-fold and the sensitivity to GABA was increased by ~10 fold for $\alpha4^{T300I}\beta2\delta$ vs wild-type receptors (Figure 1). Furthermore, the maximum estimated open probability was increased ~18-fold, showing an increased ability of GABA to gate variant $\alpha4^{T300I}\beta2\delta$ receptors. Like Vogel et al.[1] we observe that variant receptors display faster desensitization kinetics than wild-type receptors at high GABA concentrations (data not shown); however, α4β2δ receptors are extrasynaptic receptors that respond to low concentrations of GABA in the brain, and no obvious desensitization was observed with GABA concentrations below 1 μM. Thus despite the inherent complexity of receptor desensitization kinetics, we conclude that the increases in current amplitudes and sensitivity to GABA caused by the *GABRA4* variant bestow extrasynaptic δ-containing receptors with gain-of-function properties.

Of interest, the *GABRA4* Thr300 amino acid position appears to be a hotspot for pathogenic variants in most if not all $GABA_AR$ subunit classes. Besides the paralog *GABRD* p.Thr291Ile variant mentioned above,[10] we recently described the functional consequence of the paralogous variant in *GABRB3* p.Thr287Ile.[12,13] *GABRB3* encodes the β3 subunit, and we observed that this epilepsy-associated variant also causes strong gain-of-function traits. Hence, a threonine to isoleucine substitution in this specific protein position appears to cause gain-of-function traits irrespective of the subunit type.

When comparing the clinical manifestations, there are similarities as well as differences between the carriers of the *GABRA4* variant[1] and the paralog *GABRD* and *GABRB3* variants.[10,12] The *GABRA4* variant, which was observed in mosaic state (17%) in a 5.5-year-old girl, was associated with intractable nocturnal frontal lobe seizures (onset 3.5 years), dyspraxia, and attention deficit.[1] In comparison, the *GABRD* variant was observed to cause early-onset (1–4 years) generalized epilepsy with intractable atypical absence seizures, various degrees of learning difficulties/intellectual disability, and attention-deficit/hyperactivity disorder (ADHD) in a mother and her twin sons.[10] Finally, the *GABRB3* variant was observed in a child with an unclassified developmental and epileptic encephalopathy (onset 3 months) with intractable tonic,

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

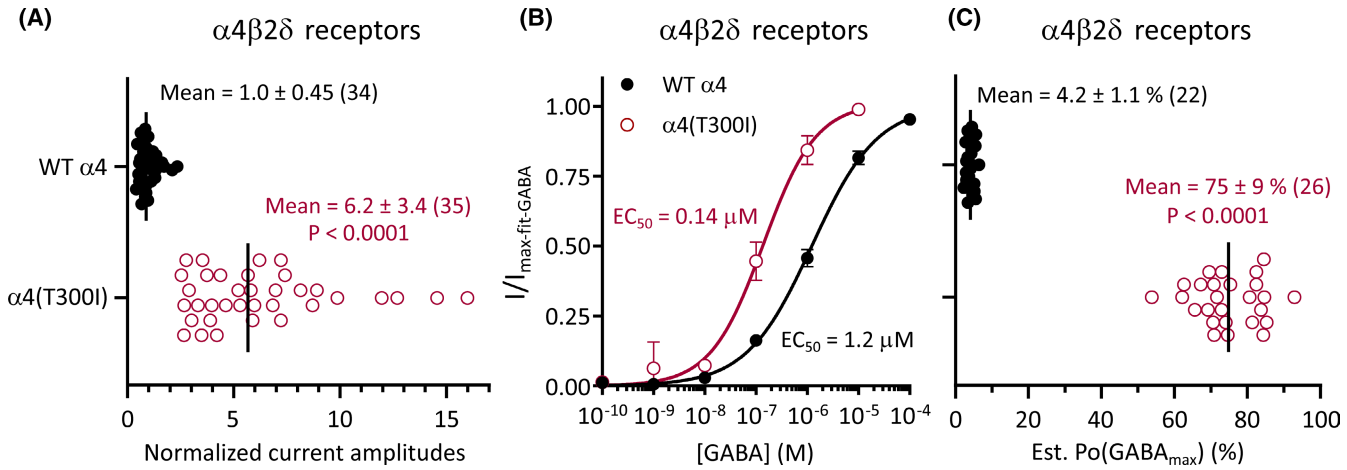

FIGURE 1 Functional analysis of the *GABRA4* p.Thr300Ile variant in α4β2δ receptors. *Xenopus laevis* oocytes were injected with complementary RNA mixtures of free α4, β2, and δ subunits in a 5:1:5 ratio and subjected to two electrode voltage-clamp electrophysiology as described previously.[10,11] (A) γ-aminobutyric acid $(GABA)_{max}$–evoked peak-current amplitudes are depicted normalized to the mean value of the wild-type receptor for each experimental day. Average values are presented as mean ± standard deviation (SD) for the indicated number of individual biological replicates and data sets were significantly different ($p < .0001$, Mann–Whitney *U* test). (B) GABA-evoked peak current amplitudes are depicted as mean ± SD as a function of the GABA concentration for $n = 8$–10 experiments for the indicated receptors. A Hill equation was fitted to the data using nonlinear regression and fitted half maximal effective concentration (EC_{50}) values are indicated in the panel. (C) Estimated open probabilities were evaluated as described previously[10,11] by comparing the response of $GABA_{max}$ (100 µM) to the response of $GABA_{max}$ in combination with a cocktail of positive allosteric modulators (allopregnanolone (3.16 µM), etomidate (31.6 µM), and Delta Selective compound 2 (DS2, 10 µM)). Data are depicted with indication of mean values ± SD for the indicated number of biological replicates and were significantly different ($p < .0001$, Mann–Whitney *U* test).

myoclonic, and focal motor seizures and severe global developmental delay.[12,13] This patient was hypersensitive to vigabatrin, and we speculate that this drug should be avoided in patients with *GABRD* and *GABRA4* gain-of-function variants as well.

Overall, our observations complement the study by Vogel et al.[1] and highlight an important role for gain-of-function extrasynaptic receptors in the etiology of epilepsy and neurodevelopmental disorders. The challenge now is to accumulate enough variants in the *GABRA4* and *GARBD* genes to robustly define the clinical phenotype that is associated with distinct functional changes in each gene.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data associated with this study are present in the paper.

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REFERENCES

1. Vogel FD, Krenn M, Westphal DS, Graf E, Wagner M, Leiz S, et al. A de novo missense variant in GABRA4 alters receptor function in an epileptic and neurodevelopmental phenotype. Epilepsia. 2022;63(4):e35–41.
2. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA(a) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience. 2000;101(4):815–50.
3. Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, et al. GABAA receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. Proc Natl Acad Sci U S A. 2006;103(41):15230–5.
4. Ahring PK, Bang LH, Jensen ML, Strobaek D, Hartiadi LY, Chebib M, et al. A pharmacological assessment of agonists and modulators at alpha4beta2gamma2 and alpha4beta2delta GABAA receptors: the challenge in comparing apples with oranges. Pharmacol Res. 2016;111:563–76.
5. Hartiadi LY, Ahring PK, Chebib M, Absalom NL. High and low GABA sensitivity alpha4beta2delta GABA receptors are expressed in Xenopus laevis oocytes with divergent stoichiometries. Biochem Pharmacol. 2016;103:98–108.
6. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(a) receptors. Nat Rev Neurosci. 2005;6(3):215–29.
7. Cope DW, Hughes SW, Crunelli V. GABAA receptor-mediated tonic inhibition in thalamic neurons. J Neurosci. 2005;25(50):11553–63.
8. Pierce SR, Senneff TC, Germann AL, Akk G. Steady-state activation of the high-affinity isoform of the α4β2δ GABA(a) receptor. Sci Rep. 2019;9(1):15997.
9. Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML, et al. Enhanced tonic GABAA inhibition in typical absence epilepsy. Nat Med. 2009;15(12):1392–8.
10. Ahring PK, Liao VWY, Gardella E, Johannesen KM, Krey I, Selmer KK, et al. Gain-of-function variants in GABRD reveal a novel pathway for neurodevelopmental disorders and epilepsy. Brain. 2022;145(4):1299–309.
11. Liao VWY, Chebib M, Ahring PK. Efficient expression of concatenated α1β2δ and α1β3δ GABA(a) receptors, their pharmacology and stoichiometry. Br J Pharmacol. 2021;178(7):1556–73.
12. Absalom NL, Liao VWY, Kothur K, Indurthi DC, Bennetts B, Troedson C, et al. Gain-of-function GABRB3 variants identified in vigabatrin-hypersensitive epileptic encephalopathies. Brain Commun. 2020;2(2):fcaa162.
13. Absalom NL, Liao VWY, Johannesen KMH, Gardella E, Jacobs J, Lesca G, et al. Gain-of-function and loss-of-function GABRB3 variants lead to distinct clinical phenotypes in patients with developmental and epileptic encephalopathies. Nat Commun. 2022;13(1):1822.