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 α4\(^{T300I}\)β2δ 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 α4\(^{T300I}\)β2δ 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\(_A\)R 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,
myoclonic, and focal motor seizures and severe global developmental delay.\textsuperscript{12,13} This patient was hypersensitive to vigabatrin, and we speculate that this drug should be avoided in patients with \textit{GABRD} and \textit{GABRA4} gain-of-function variants as well.

Overall, our observations complement the study by Vogel et al.\textsuperscript{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 \textit{GABRA4} and \textit{GABRD} genes to robustly define the clinical phenotype that is associated with distinct functional changes in each gene.

**FUNDING INFORMATION**


**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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**FIGURE 1** Functional analysis of the \textit{GABRA4} p.Thr300Ile variant in \textit{α4β2δ} receptors. \textit{Xenopus laevis} oocytes were injected with complementary RNA mixtures of free \textit{α4}, \textit{β2}, and \textit{δ} subunits in a 5:1:5 ratio and subjected to two electrode voltage-clamp electrophysiology as described previously.\textsuperscript{10,11} (A) \(\gamma\)-aminobutyric acid (GABA\textsubscript{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\textsubscript{50}) values are indicated in the panel. (C) Estimated open probabilities were evaluated as described previously\textsuperscript{10,11} by comparing the response of GABA\textsubscript{max} (100 \(\mu\)M) to the response of GABA\textsubscript{max} in combination with a cocktail of positive allosteric modulators (allopregnanolone (3.16 \(\mu\)M), etomidate (31.6 \(\mu\)M), and Delta Selective compound 2 (DS2, 10 \(\mu\)M)). 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).

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