GABRA1-related disorders : from genetic to functional pathways

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Running head: *GABRA1* genotype-phenotype correlations

Summary for Social Media If Published

Variants in *GABRA1* have been associated with a broad epilepsy spectrum, however our understanding of what determines the phenotype severity and best treatment options remains inadequate. In this collaborative work, we aimed to analyse the electro-clinical features of 27 unpublished individuals harbouring 20 different *GABRA1* variants and to explore functional effects of 19 variants.

Our genotype-phenotype correlations permit us to delineate specific sub-phenotypes for LoF and GoF variants, with different epileptic and neurodevelopmental features. Going forward, this study will pave the way for a better understanding of the patho-mechanism of *GABRA1*-related disorder and for a precision medicine approach.

Draft Tweet

Investigation of 27 novel patients expand the field of *GABRA1*-related neurological disease and highlights the importance of functional analysis and genotype-driven deep phenotyping

ABSTRACT

Objective:

Variants in *GABRA1* have been associated with a broad epilepsy spectrum, ranging from genetic generalized epilepsies to developmental and epileptic encephalopathies. However, our understanding of what determines the phenotype severity and best treatment options remains inadequate. We therefore aimed to analyse the electro-clinical features and the functional effects of *GABRA1*-variants to establish genotype-phenotype correlations.

Methods:

Genetic and electro-clinical data of 27 individuals (22 unrelated and 2 families) harbouring 20 different *GABRA1* variants were collected and accompanied with functional analysis of 19 variants.

Results:

Individuals in this cohort could be assigned into different clinical subgroups based on the functional effect of their variant and its structural position within the *GABRA1* subunit. A homogenous phenotype with mild cognitive impairment and infantile-onset epilepsy (focal seizures, fever sensitivity and EEG posterior epileptiform discharges) was described for variants in the extra-cellular domain and the small transmembrane loops. These variants displayed loss-of-function (LoF) effects and the patients generally had a favourable outcome. A more severe phenotype was associated with variants in the pore-forming transmembrane helices. These variants displayed either gain-of-function (GoF) or LoF effects. GoF-variants were associated with severe early-onset neurodevelopmental disorders, including early infantile developmental and epileptic encephalopathy.

Interpretation:

Our data expand the genetic and phenotypic spectrum of *GABRA1*-epilepsies and permit to delineate specific sub-phenotypes for LoF and GoF variants, though the heterogeneity of phenotypes and variants. Generally, variants in the transmembrane helices cause more severe phenotypes, in particular GoF variants. These findings establish the basis for a better understanding of the patho-mechanism and precision medicine approach in *GABRA1*-related disorders. Further studies in larger populations are needed to provide a conclusive genotype-phenotype correlation.

Keywords: *GABRA1, neonatal encephalopathy, infantile epilepsy, febrile seizures, juvenile myoclonic epilepsy*

1. Introduction

The *GABRA1* gene is located on chromosome 5 and encodes the α1 subunit of the gamma-aminobutyric acid type A receptor ($GABA_AR$). $GABA_ARs$ are pentameric chloride channels assembled from 1–5 of 19 subunits. The most common $GABA_AR$ expressed in the mammalian brain is composed of two α1, two β2/β3 and one γ2 subunits (Figure 1A & 1B). Each subunit shares common structure, consisting of (i) an extracellular N-terminus domain, (ii) a transmembrane domain (TM) made up of four transmembrane helices (TM1–4) and two loops TM1-TM2 and TM2-TM3, and (iii) an intracellular domain (intracellular loop TM3-TM4)[1].

$GABA_AR$ mediate crucial inhibitory synaptic transmission in the central nervous system (CNS)[2]. Pathogenic variants in *GABRA1* were first identified in unrelated individuals and families with genetic generalized epilepsies, including juvenile myoclonic epilepsy (JME)[3 4] and childhood absence epilepsy[5]. More recently, *GABRA1* variants have been reported in severe developmental and epileptic encephalopathies (DEE)[6], including Dravet-like phenotypes[7 8 9], Ohtahara Syndrome, epilepsy of infancy with migrating focal seizures (EIMFS) and infantile spasms syndrome (ISS)[10 11 12 13]. Overall, this represents a very diverse phenotypic spectrum.

While variants in *GABR* genes can be observed along the entire length of the subunit, they are typically enriched in paralog-conserved sites with clustering around key functional structures such as the extracellular GABA binding site, transmembrane helices supporting or lining the pore, and loop regions that couple ligand binding to channel gating. Functional analysis of variants, including *GABRA1* variants, traditionally reported only loss-of-function (LoF) effects. Variants were found to reduce or abolish GABAergic function by either lowering the channel gating efficiency or receptor surface expression, thereby limiting inhibitory GABAergic transmission resulting in epileptic phenotypes[14]. Phenotypic severity has then been speculated to be a result of the degree of LoF traits[15]. However, the assumption of the same functional effect of all variants (LoF) in a gene such as *GABRA1* does not correlate well with the unusual diverse range of severities in clinical phenotypes.

We recently reported that the phenotypic diversity associated with epilepsy-causing *GABRB3, GABRA4* and *GABRD* variants is not only explained by LoF but can also be explained by the presence of variants displaying a GoF effect[16 17 18 19]. These GoF $GABA_AR$ variants were associated with treatment resistant epilepsy and typically more severe phenotypes. Almost all *GABRA1* variants tested to date have been reported as LoF[14], but recently one p.(Thr292Ser) was reported as GoF[20] and another one p.(Ala332Val) was inferred to have GoF traits[21]. Given that $GABA_AR$ α1 subunits structurally resemble β3 subunits and assemble into the same receptor complexes, we speculated that historic data might not fully reflect the consequences of *GABRA1* variants. Therefore, in this study we present a cohort of 27 novel individuals carrying 20 (11 novel) presumed pathogenic *GABRA1* variants and aimed to establish possible explanations

for the heterogeneity of the phenotypic spectrum using deep phenotyping and EEG characterisation coupled with functional analysis of each variant.

2. Materials and Methods

Ethics

All institutions involved in human participant research received local IRB approval (main IRB: The ethics committee of Region Zealand, Denmark). Written informed consent, including authorization for reproduction of video images, was obtained for all patients (or legal guardians) and family members where necessary. Patient data were collected according to local ethics committee guidelines.

Data collection and analysis

Through an international collaboration including different clinical epilepsy centres in Europe, Australia and in the United States, we collected data of individuals with presumed pathogenic *GABRA1* variants. The American College of Medical Genetics and Genomics/Association of Molecular Pathology guidelines were used to assess variant pathogenicity and the *GABRA1* transcript NM_000806.5 to code variant nomenclature. Clinical information was collected by face-to-face interviews with patients and their families and from clinical charts, and data have been collected in a structured phenotyping table. The epilepsy syndromes were classified according to the guidelines of ILAE classification (2017)[22]. All the EEG and cerebral MRI reports were collected. Two epileptologists with EEG expertise (E.M., E.G.) reviewed the raw EEG data of 15 patients (including long-term monitoring video-EEGs) for background activity, interictal epileptiform abnormalities, ictal EEG discharges and clinical manifestations.

Functional studies

α1β3γ2 $GABA_A$ receptors have a pentameric stoichiometry consisting of two α1, two β3 and one γ2 subunits. All patients in this cohort are heterozygous for their variant. Assuming a Mendelian distribution for the missense variants, this results in 50% of the expressed receptors having a single variant α1 subunit, 25% of expressed receptors having two variant α1 subunits, while the remaining 25% of expressed receptors are wild-type. Hence, the most important receptors to investigate are the ones that are heterozygous for the variant, as these constitute the bulk of expressed receptors. Pentameric concatenated constructs represent a highly efficient method to obtain robust expression of such receptors in *Xenopus laevis* oocytes[23 24].Thus, we generated 19 pentameric concatenated constructs containing a variant and a

wild-type α1 subunit (Figure 2A). For functional analysis, the cRNAs of concatenated α1β3γ2 variant receptors and the wild-type (control) were injected into oocytes (25 ng/oocyte) and subjected to electrophysiological investigation using the two-electrode voltage clamp technique as previously described[23]. Functional electrophysiological experiments were conducted using a custom-built two-electrode voltage-clamp apparatus. Inter-day variation between oocyte batches were controlled by performing wild-type experiments in parallel to variants on each experimental day. GABA concentration–response relationships (n > 10) were obtained by applying increasing concentrations of GABA. Maximum current amplitudes (I_{max}, n > 20) were determined by applications of 10 mM GABA solution and normalized against wild-type value of the day. All experiments were conducted in at least two batches of oocytes.

To obtain the EC_{50} values from the GABA concentration–response relationships, the Hill equation was fitted to the GABA-evoked current amplitudes for individual oocytes where EC_{50} is the concentration eliciting half-maximum response, and nH is the Hill slope:

$$I = I_{max}\left(\frac{[\mathrm{GABA}]^{nH}}{[\mathrm{GABA}]^{nH} + \mathrm{EC_{50}}^{nH}}\right)$$

Responses were then normalized to the fitted maximum response of individual curves. Full concentration-response curve of individual oocytes was recorded as a single determination (n). Average pEC_{50} values (where p = -LOG) for the wild-type control (pEC_{50}(wt)) was calculated and the ΔpEC_{50} values for each variant experimental determination on the same day was derived by the equation:

$$\Delta\mathrm{pEC_{50}} = \mathrm{pEC_{50}} - \mathrm{pEC_{50}(wt)}$$

Typically, in this assay a ΔpEC_{50} value of ≥ 0.2 indicates a GoF and ≤ -0.2 a LoF variant. The higher the number towards either the positive or negative spectrum the more altered receptor sensitivity to GABA.

3. Results

Patients and genetics

We collected data from 27 individuals (22 unrelated, and 2 families) harbouring 20 presumed pathogenic *GABRA1* variants of which 11 are novel. All variants were heterozygous missense, except for 1 frameshift p.(Gly222Aspfs*4). The variants occurred *de novo* in 15/27 (55%) subjects and were inherited from a symptomatic parent in 6/27 (22%), with high penetrance. Segregation data were not available for 6 subjects.

17/20 variants were predicted as damaging by at least two different prediction tools (SIFT, PolyPhen, MutationTaster). The missense variants were distributed among the various domains of the α1 subunit protein: 9 in the extracellular domain, 9 in the transmembrane domain and 1 in the intracellular domain

(that is not resolved in the Cryo-EM structures of the $GABA_AR$) (Figure 1C & 1D). The frameshift variant (Gly222Aspfs*4) was located in the extracellular domain.

Functional analysis of GABRA1 variants in α1β3γ2 receptors

As described in the methods, functional analysis was performed on receptors that are heterozygous for the *GABRA1* variants (Figure 2A). Wild-type receptors and receptors containing missense variants all responded to GABA applications in a concentration-dependent manner (Figure 2B & 2C). However, in most cases the sensitivity to GABA was different from that of the wild-type receptor (Figure 2D). Four variants (p.(Tyr252His), p.(Ser299Asp), p.(Ala332Thr) and p.(Tyr438Cys)) had significantly increased sensitivity to GABA, which constitute a GoF effect (Figure 2D, Table 1). In contrast 12 variants displayed decreased sensitivity to GABA, which constitute a LoF effect. The remaining three variants (p.(Glu63Lys), p.(Ser96Cys) and p.(Glu403Gln)) did not display any significant difference relative to the wild-type receptor. Next, maximally GABA-evoked current amplitudes were assessed to investigate whether the variants might affect surface levels or gating efficiency of expressed receptors. Of the 19 tested variants, only the p.(Val270Ala) variant displayed significantly lower maximal current amplitude (Figure 2B, Table 1). As this variant also displayed LoF for the GABA sensitivity, this variant essentially displays LoF traits for both investigated parameters.

Reclassification of variants using the ACMG guidelines

The p.(Gly222Aspfs*4) variant will likely lead to nonsense mediated decay of the mRNA and limited or no protein synthesis. In case the protein is expressed, the synthesis would terminate early leading to a partial subunit that is missing the entire transmembrane domain and therefore is non-functional. This variant can be classified as likely pathogenic according to the American College of Medical Genetics (ACMG) guidelines[25] based on a null variant in a gene where LoF is a known mechanism of disease (PVS1). Of the remaining 19 tested variants, 12 variants were confirmed to occur *de novo* (PS1) and show deleterious effects in a well-established assay (PS3), which leads to a classification as pathogenic (Table 1). Two variants [p.(Asn275Lys) and p.(Pro305Leu)] were inherited from an affected parent and showed deleterious effects in the functional assay leading to a likely pathogenic classification. For two variants [p.(Arg147Gln) and p.(Val162Met)], the segregation status was not confirmed but deleterious effects were noted in the functional assay leading to a likely pathogenic classification.

Finally, the 3 variants [p.(Glu63Lys), p.(Ser96Cys) and p.(Glu403Gln)] that were predicted to not be damaging using *in silico* tools, did not display any deleterious effects in the functional assay. These variants have been reported in 3 unrelated subjects with different phenotypes: (1) one, with intellectual disability

(ID) and generalised epilepsy, inherited the variant p.(Glu63Lys) from the affected father; (2) one, with generalised epilepsy and severe ID, inherited the variant p.(Ser96Cys) from the asymptomatic mother; (3) the last one, with childhood absence epilepsy, harbours a *de novo* p.(Glu403Gln) variant. Although it is not possible to exclude a potential pathogenic cause that were missed with our experimental system, it is likely that these represent benign polymorphisms not responsible for the clinical phenotype of the individuals. This is supported by the observation that two of these three amino acid positions have entries in gnomAD database [p.(Glu63Ala), p.(Glu403Gln) and p.(Glu403Lys)]. The 3 patients have therefore been excluded from our genotype-phenotype analysis.

GABRA1 cohort

The remaining 24 individuals (15 females / 9 males), included for the genotype-phenotype analysis encompass 21 probands and 3 affected family members. Their age at inclusion ranged from 12 months to 34 years (median age 8.5 years). We investigated the clinical phenotype with a focus on epilepsy, EEG features and the cognitive and neurological outcomes (Table 2). Comprehensive electro-clinical and genetic details of the whole cohort are summarized in the supplementary table.

Epilepsy was diagnosed in 23/24 (96%) subjects, with a median age at onset of 9.5 months (range: 1.5 month - 15 years). Seizure types included focal and generalized tonic-clonic seizures, typical and atypical absences, myoclonic and atonic seizures, and epileptic spasms. Most individuals (21/24, 88%) had various degrees of ID or developmental delay. Other neurodevelopmental disorders were observed: autism spectrum disorder (ASD) (5/24, 21%) and behavioural disturbances or attention deficit/hyperactivity (ADHD) (7/24, 29%). Movement disorders, including ataxia, poor coordination, tremor, dystonia, and cerebral palsy were also reported (7/24, 29%).

Genotype-phenotype correlations

Functionally, the 7 missense variants located in extracellular domain had a LoF effect. In the transmembrane domain, we observed 4 missense variants with GoF effect and 5 variants with LoF effect of which two were inherited. Apart from the inherited variants, the variants in the transmembrane domain generally caused more severe and varied phenotypes compared to the variants in the extracellular domain. Based on the position and on the functional effect of the missense variants, we distinguished different subgroups with different phenotypic features (Table 2, Figure 3).

Extracellular domain missense variants (LoF)

11 individuals, aged between 1 to 19 years, harboured 7 missense variants in the extracellular domain. The modality of inheritance was *de novo* in 8. The segregation analysis was not available for 3 individuals. All subjects showed a homogenous phenotype.

Mild-to-moderate ID was described in 9/11 (82%) and severe ID in 1. Early motor milestones were slightly delayed in 7/11 (64%), however all subjects reached normal motor autonomy, and achieved independent walking within the age of 24 months. Early language development delay was reported for 9/11 (82%) subjects; 10 reached afterword normal verbal skills, while one (9 years old) was a nonverbal child with ASD. Pyramidal or extrapyramidal signs were not observed; broad-based ataxic gate, poor coordination and mild hypotonia were reported in 8/11 (73%) during infancy / early childhood, with progressive improvement.

Comorbid neuropsychological and neuropsychiatric conditions included ASD in 3/11 (27%), behavioural dysregulation in 2/11 (18%) and hyperactivity in 1/11 (9%). All the patients were at least partially autonomous in everyday life, apart from a child with ASD who showed poor independence in daily activities, personal and social autonomy.

Epilepsy was reported in all, typically with onset in the first year of life (9/11, 82%) and with focal hemiclonic seizures (6/11, 55%) elicited by high fever in 4 of them. Less frequently, brief febrile generalized tonic-clonic (GTC) (3/11, 27%), or myoclonic seizures (1/11, 9%) were observed at onset. During late-childhood, focal or focal-to-bilateral tonic clonic seizures were observed in 7/11 (64%), while GTC and myoclonic seizures, including eyelid myoclonia, in 5/11 (46%). Fever sensitivity was reported in 9/10 (90%) individuals (data not available for 1). After a “stormy phase”, 6/11 (55%) subjects became seizure-free on monotherapy at an age ranging from 9 months to 6 years, while 4/11 (36%) achieved partial seizure control on 1-2 anti-seizure medications (ASM), with a maximum seizure frequency of 1 seizure/month. Only 1 subject had severe epilepsy with the need for polytherapy. LEV was the most effective drug (8/8 subjects who tried the drug), although 2 experienced negative behavioral side effects. Benzodiazepines were not tested in this group.

Interictal EEG was normal at epilepsy onset in in 4/7 (57%) and showed focal (parieto-temporal or occipital) interictal epileptiform discharges (IED) with activation during sleep in 3/7 (43%) (data not available for 4 individuals). At follow up, intermittent slow background with diffuse superimposed fast rhythms or excess of beta activities in the central regions were recurrent findings (3/9, 33%; data not available for 2 patients). No IED were observed in 6/9 (66%), while 2/9 (22%) subjects had posterior spikes with tendency to diffuse spreading and sleep activation, and 1/9 (11%) had diffuse spike-and-slow waves (Figure 4A).

A syndromic classification of genetic epilepsy with febrile seizures plus (GEFs+) was made for most patients, because of onset with febrile seizures in the first year of life and afebrile polymorphic seizures appearing later in life, with some atypical features (e.g. mild ID). For some patients, Dravet syndrome (DS) was

suspected, as previous normal infants presented with prolonged, febrile, hemiclonic seizures followed by developmental slowing. However, prolonged seizures and status epilepticus were not common in this cohort, gait abnormalities and motor impairment were non-progressive, and an encephalopathic course was not observed. To the opposite, complete seizure control on monotherapy was achieved. EEG are normal or with an occipital focus with activation during sleep (similar to focal idiopathic epilepsy). For these reasons, the developmental course does not underpin the clinical diagnosis of DS.

Transmembrane domain missense variants (LoF)

Three individuals harbouring LoF variants located in the TM1-TM3 helices encompassed a range of neurodevelopmental disorders associated with early-onset epilepsy with prominent photosensitivity.

Pt.15 [p.(Val270Ala), TM1] had severe hypotonia and global developmental delay as an infant; she never sat nor walked independently She developed focal seizures at 1.5 months of age, and at 14 years multi-focal myoclonus that has increased over time. After multiple ASM trials, seizures were ultimately controlled with primidone, started for kinetic tremor. At latest control (16 years old), she had severe ID, behavioural dysregulation and generalized hypotonic-dystonic cerebral palsy (GMFCS level 5). Comorbidities included dysphagia and poor weight gain, treated by gastrostomy tube placement. EEG showed slow background, abundant beta activity and photosensitivity; at latest follow-up continuous delta slowing, and superimposed beta activity was described.

Pt.19 [p.(Thr295Ile), TM2] presented with developmental delay from early infancy and focal hemiclonic seizures from the age of 7 months, followed by focal autonomic seizures. After a “stormy phase”, he achieved seizure control on LEV from the age of 16 months. Epilepsy relapsed at 6 years with recurrent unprovoked absences with eyelid myoclonia. EEG at onset was normal, then showed occipital and diffuse spike-and-waves and photosensitivity.

Pt.22 [p.(Ile317Leu), TM3]_is a 17 months old boy, with hypotonia, developmental and motor delay from early infancy, and GTC from the age of 7 months. Longer follow up is needed to determine epilepsy and cognitive outcome. Long-term monitoring EEG showed slow background and excess of beta activity in the central regions (Figure 4B).

Individuals harbouring inherited LoF-variants located in TM1-TM2 and TM2-TM3 loops presented with milder phenotypes similar to the ones carrying variants in the extracellular domain. Photosensitivity seems to be a common feature during late childhood/adolescence.

Pt. 16-18 [p.(Asn275Lys), TM1-TM2 loop] were members of a GEFs+ family, sharing a quite similar phenotype. All 3 had mild ID, normal behaviour and normal neurological examination. All 3 started having

febrile seizures within 18 months of life, followed by afebrile GTC (proband and mother) and typical absences (sister) during infancy. The interictal EEG initially showed focal IED in the posterior regions, then diffuse spike and slow waves at follow up (Figure 4C). All 3 achieved seizure-freedom on LEV, however seizures with photosensitivity relapsed in the mother from the age of 12 until 30 years (good control on carbamazepine).

Pt.21 [p.(Pro305Leu], TM2-TM3 loop, inherited from a symptomatic father), is a 10-year-old girl with mild ID, learning disabilities, normal behaviour and normal neurological examination. She presented with childhood onset epilepsy; Panayiotopoulos syndrome was suspected, due to the age of epilepsy onset (4 years), the seizure semiology (prolonged focal autonomic seizures), and the posterior spike-and-waves with photosensitivity.

Transmembrane domain missense variants (GoF)

The 4 individuals included in this group encompasses a range of severe clinical phenotypes, ranging from infantile spasms syndrome (ISS) to isolated neurodevelopmental disorders without epilepsy.

Pt.14 [p.(Tyr252His), TM1] presented with infantile spasms, developmental regression and hypsarrhythmia at the age of 5 months. Infantile spasms were responsive to ACTH and prednisolone and the patient achieved seizure freedom, although the persistence of almost continuous EEG abnormalities, treated with VPA, LEV and sulthiame with no benefit. His EEG showed multifocal IED (4 year old), fronto-temporal IED (7 year old) and was normal by the age of 9 years. His motor development was delayed, but no seizures were reported at follow up. At the age of 18 years, he was ambulant, but still presented with impairment of speech competencies (severe language disorder), cognitive abilities (moderate ID) and behaviour regulation.

Pt.20 [p.(Ser299Asn), TM2] presented with severe ID, movement disorders and autism, but never had seizures nor IED on the EEG. However, since she is only 3 years and 6 months old, a longer follow up is needed to define her phenotype.

Pt.23 [p.(Ala332Thr), TM3] presented with global developmental delay from early infancy, evolving to moderate ID associated with mild dystonic posturing, pyramidal signs, and poor language. GTC occurred at the age of 10 years and were not completely controlled by VPA.

Pt.24 [p.Tyr438Cys, TM4] had developmental delay evolving to mild ID, ASD and ADHD with anxiety disorder. Myoclonic seizures had onset at the age of 27 months, and subsequently focal motor seizures and atypical absences appeared (5 years old). At latest control (8 years old), he still had seizures despite

multiple ASM (VPA, clobazam, rufinamide, lamotrigine). The EEG showed posterior or diffuse slowing and generalized 2-3 Hz spike-and-slow waves (Figure 4D).

Frameshift variant

The only protein truncating variant [p.(Gly222Aspfs*4)] was observed in a proband and her mother (pt.10 and pt.11), both with pharmacoresponsive epilepsy, normal neurological examination and normal cognition. The syndromic classification is difficult because epilepsy showed both features of genetic generalized epilepsy as well as of focal epilepsy. The mother had two GTC during adolescence, whilst her daughter had adolescence-onset focal seizures (autonomic manifestations, automatisms, staring) and GTC, well controlled with carbamazepine. The EEG of the daughter showed sporadic IED in the frontal region bilaterally, and myoclonic seizures with generalized spike-and slow waves.

4. Discussion

In this study, we defined the electro-clinical features of 24 novel patients harbouring 17 different disease-causing *GABRA1* variants including 11 novel variants. We distinguished different electro-clinical phenotypes and described the complex genotype-phenotype correlations. Our data confirm the broad clinical spectrum of *GABRA1,* encompassing both epilepsy and movement disorders[26], including pyramidal and extra-pyramidal motor impairment. Interestingly, the different *GABRA1* phenotypic expression depends on the type (missense vs nonsense), the position (extracellular vs transmembrane domains) and ultimately on the functional effect (LoF vs GoF) of the variants (Figure 3). The combined impact of these three aspects, appear to define the overall clinical features including the severity of epilepsy, cognitive and motor development.

Functional analysis

The 19 *GABRA1* missense variants were functionally evaluated as α1β3γ2 $GABA_A$ receptors in heterozygous assemblies containing one variant and one wild-type *GABRA1* subunit. 12 variants displayed LoF traits, and 4 variants displayed GoF traits which enables classifications of likely pathogenic or pathogenic according to the ACMG guidelines. The remaining 3 missense variants did not cause any significant functional change and likely represent benign variants. Based on multiple entries in the gnomAD database, one of these three variants, p.(Glu403Gln), might have been excluded from the study onset, however, inclusion of expected benign variants is critical when performing functional analysis. This validates that the assay can be used to distinguish between the three categories of GoF, LoF and benign variants[27]. Historically, epilepsy-associated

GABR variants were all assumed to be LoF[15], but the data presented here add the *GABRA1* subunit to a growing list of *GABR* subunits for which both LoF and GoF variants have been confirmed. This list currently includes *GABRA4*[18], *GABRB3*[16 17]and *GABRD*[19]. In the current study, LoF variants were found in both the extracellular and transmembrane domains while GoF variants were only found in the transmembrane helixes. While this suggests that extracellular domain *GABRA1* variants are more likely to display LoF traits, GoF variants could still emerge once more variants are functionally characterised.

Frameshift variant

Protein truncating *GABRA1* variants are not commonly described, with only three previously reported cases: 1 deletion-insertion in a family with genetic generalized epilepsy[4], 1 patient with childhood absence epilepsy[5], and 1 case in comorbidity with Williams-Beuren Syndrome[28]. Apart from the comorbidity, protein truncating variants seem to be related to normal cognition and mild, late-onset epilepsy with a good outcome. This is also what we observed for the single frameshift variant [p.(Gly222Aspfs*4)] in this study (Figure 3). From a protein function perspective, protein truncating variants can cause nonsense-mediated mRNA degradation[29] and/or protein truncation, hence they are expected to lead to no protein or non-functional proteins, which ultimately are not incorporated into a pentameric receptor complex. These variants are thus *de facto* LoF and resemble haploinsufficiency. It is quite likely that upregulation of expression of the subunit using the healthy gene copy leads to some degree of compensation, however, it is still noteworthy that haploinsufficiency with up to 50% of normal receptor expression leads to epilepsy with a relatively mild and treatable phenotype.

Missense extracellular domain variants

Missense variants located in the extracellular domain were all found to have LoF effect and presented with a homogenous phenotype, with seizure onset in the first year of life in the majority of cases (82%), typically with focal hemiclonic seizures elicited by high fever (Figure 3). Fever sensitivity is a hallmark for this group throughout their epilepsy course, and it is particularly remarkable for some specific variants [p.(Arg112Gln), p.(Arg214His)]. These features are similar to what has been previously described for *GABRA1* patients with syndromic classification of Dravet syndrome, Dravet syndrome-like, early-onset epileptic encephalopathy and GEFs+[6 7 8 9 12 13].However, the cognitive and epilepsy outcomes for this group were much better than what is expected for Dravet syndrome or for most early-onset DEE. During infancy, they typically developed mild-to-moderate ID and focal autonomic seizures and eventually focal-to-bilateral tonic-clonic or myoclonic seizures, achieving seizure freedom on ASM monotherapy. Prolonged seizures and status epilepticus were exceptional and an encephalopathic course was never observed. The EEG pattern of this

group consists of focal posterior slowing and IED, and diffuse spike-and-waves. This is also clearly distinguishable from the EEG of Dravet syndrome[30]. While formally not part of the extracellular domain, LoF variants located in the transmembrane loops TM1-TM2 or TM2-TM3 overall resembled the extracellular domain group.

Missense transmembrane helices variants

Missense variants located in the transmembrane helixes were associated with more severe phenotypes. These observations align well with previously reported cases, where *GABRA1* variants with the most severe phenotypes (*e.g.,* early-onset ISS, Ohtahara syndrome[10]or EIMFS[11]) were found in the TM1 and TM2 transmembrane helices. Variants in the TM1 and TM2 thus appear to share a clinical phenotype of EIDEE with early-onset epileptic spasms, evolving into severe refractory epilepsy, severe ID, and severe neurological impairment, and inauspicious outcome (Figure 3).

Interestingly, variants in helixes had either a LoF or a GoF effect. We identified four GoF variants, which represent the first description of *GABRA1*-GoF DEE [p.(Tyr252His), p.(Ala332Thr) and p.(Tyr438Cys)], whereby the phenotype mainly consist of early-onset neurodevelopmental disorder and early life epilepsy (*e.g.* ISS). The individual with the remaining GoF variant p.(Ser299Asn) also has developmental delay and severe ID, however, so far without epilepsy (current age: 42 months). This is similar to a recently reported single *GABRA1* GoF variant (p.(Thr292Ser)) in a 2-year-old subject with severe neurodevelopmental delay and no seizures [20]. LoF variants located in the transmembrane helical regions also have severe phenotypes that are not clearly distinguishable from *GABRA1*-GoF DEE, except for photosensitivity which seems to be a prominent feature of DEE GABRA1-LoF variants. Photosensitivity is not very frequent in DEEs [31]or subjects with variants in other GABR subunits[17 19]. If confirmed in a larger population, this feature could contribute to addressing the diagnosis of GABRA1-LoF DEE. The LoF variant p.(Val270Ala) located in TM1 is particularly interesting because it causes a large loss in GABA sensitivity (~10-fold) coupled with a significant loss in GABA-elicited current amplitude (~60% loss) (Table 1). While the detriment of "double losses" in protein function correlates with the severe phenotype in our patient, it is noteworthy that this variant was recently described in another subject with early-onset treatable epilepsy and moderate developmental delay [12], suggesting heterogeneity in phenotypic outcome for this variant.

GoF and LoF variants in GABR subunits

Similar to our genotype-phenotype and functional correlation studies in *GABRB3* and *GABRD*[32 19 17], GoF and LoF variants in *GABRA1* overall display distinctive phenotypic features that can be distinguished and separated into clinical categories. In all three genes, LoF variants including protein truncating variants are

generally mild, with good prognosis and can be inherited. Severe phenotypes such as DEE and Dravet-like phenotype are typically only described for LoF variants in transmembrane helixes. In contrast, GoF variants are *de novo* and are typically associated with the more severe, drug resistant epilepsy with poorer clinical outcome. Currently, our understanding of how GoF $GABA_AR$ variants cause severe neurodevelopmental disorders is limited. That said, both GoF and LoF variants have recently been described in several epilepsy-causing genes including *SCN1A, SCN8A* and *CACNA1A* indicating that this is not a unique phenomenon [33 34 35]. A commonality for all these genes is that patient phenotypes and treatment options vary significantly between GoF and LoF variants. Hence, future studies are urgently needed to unravel how the different variant types affect neurodevelopment and normal brain circuitry.

Unlike variants in *GABRB3* and *GABRD*, the individuals with GoF *GABRA1* variants in this cohort achieve seizure control with or even without ASM. This observation could simply be due to the small sample size, or it could be related to the differences in the role the different subunits play in receptor function, and regions of the brain where the resulting receptors are found. A larger sample size and more *GABRA1* variants will need to be functionally characterised to establish more solid genotype-phenotype correlations and improve our understanding of the role this subunit plays in the pathogenesis of epilepsy.

Finally, given that the degree of detrimental change in receptor function can be successfully quantified and encapsulated using our functional parameters (*e.g.,* ΔpEC50) it is tempting to speculate that disease severity is linked to the magnitude of functional change. This notion is supported by the observation that highly altered receptor function in either direction of gain or loss caused a severe phenotype, exemplified by p.(Val270Ala) and p.(Ser299Asn). However, across the cohort there is no strong linkage between the magnitude of functional change and disease severity. While this is likely related to the size of the cohort, it is also important to note that different patients with the same *GABR* variant can vary substantially in their clinical presentation.

Treatment options

Our current knowledge of identifying the best treatment option for patients with either LoF or GoF variants is poor. However we can speculate from our *GABRB3* study, that seizure freedom is more common in subjects with LoF variants, particularly for variants located in the extracellular domain. Interestingly the most effective treatment for LoF variants in *GABRA1* and *GABRB3* vary with LEV identified for *GABRA1* individuals versus VPA in *GABRB3*[32]. Conversely, ASMs are ineffective in patients with GoF *GABRA1* variants with severe epilepsy, and this was also observed for individuals harbouring pathogenic *GABRB3* variants located in TMD domain[32]. Benzodiazepines, that specifically enhance the effects of endogenous and exogenous GABA mediated by $GABA_A$ receptors, have not been tried on any of the subjects in our cohort.

5. Conclusion

It is well known that LoF variants in the *GABRA1* gene can cause epilepsy, however, here we demonstrate that variants with a GoF effect can also cause neurodevelopmental disorders, including epilepsy. This observation aligns with recent observations for *GABRB3* and *GABRD*. *GABRA1* LoF and GoF variants lead to different epilepsy phenotypes of different severity, also depending on their protein position. In general, variants in the transmembrane helices present with severe phenotypes, especially GoF variants that are associated with the most severe neurodevelopmental disorders, autistic features and early-onset epilepsy. LoF variants in the extracellular domain as well as variants in the transmembrane loop regions give raise to a quite homogenous and relatively benign phenotype (Figure 3). These findings pave the way for testing the possibility of a precision medicine approach to the treatment of *GABRA1*-epilepsies. Larger cohort studies are needed to be confirm and better quantify these results.

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Author Contributions

R.S.M., P.K.A., M.C. and E.G. contributed to the conception and design of the study. E.M., K.M.J., C.D.F., D.L., K.K., K.F., B.B., P.V., D.D., B.C., S.W., P.S., A.B., S.A.H., C.T., D.I.B., H.G., T.F., S.B., L.T., M.B., N.D., O.S., E.D., M.K., G.C., I.C., T.P., B.I., S.M.B., W.F., L.W., M.J.M., F.D., E.P., D.L.D., R.A.J., S.K., J.P., N.M., E.B. ,V.W.Y.L., P.K.A., M.C., E.G. and R.S.M. contributed to the acquisition and analysis of data. E.M., V.W.Y.L., E.G., M.C., R.S.M. and P.K.A. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data availability

De-identified data, including the *GABRA1* database and data used for functional studies, will be made available to those eligible and be stored for 10 years.

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Legends

Table 1 Fitted and recorded data for α1β3γ2 $GABA_A$ receptors containing *GABRA1* variants

Xenopus laevis oocytes were injected with cRNA for concatenated constructs containing the indicated variants and subjected to two-electrode voltage-clamp electrophysiology as described in the methods. A Hill equation was fitted to GABA concentration–response datasets by non-linear regression. The difference in fitted GABA sensitivities (EC_{50} values) for variant and wild-type receptors is presented as ΔpEC_{50} ± SD where p = –log for the indicated number (n) of individual oocytes. Analysis for statistical significance was obtained using One-way ANOVA with *post hoc* Dunnett's test relative to the wild-type receptor. The average maximal current obtained with $GABA_{max}$ (10000 µM) applications is presented as $GABA_{max}$ ± SD in nA for the indicated number (n) of individual oocytes. Analysis for statistical significance was obtained using Mann-Whitney U-test comparing variant to wild-type receptor data from the same experimental days. For both statistical analysis, significance is claimed when $P < 0.0001$.

Table 2 Characteristics of the *GABRA1* subgroups

Abbreviations: Ab: absences, aAb: atypical absences, ACTH: adrenocorticotropic hormone, ADHD: Attention Deficit / Hyperactivity Disorder, ASD: autism spectrum disorder, Behav: behavioral, CBZ: carbamazepine, DE: developmental encephalopathy, DEE: developmental and epileptic encephalopathy, dist.: disturbance, FE: focal epilepsy, FS: febrile seizures, GEFs+: genetic epilepsy with febrile seizures plus, GoF: gain of function, GTC: generalized tonic clonic, ID: intellectual disability, IGE: Idiopathic Generalized Epilepsies, IS: infantile spasm, LEV: levetiracetam, LoF: loss of function, mod.: moderate, My: myoclonic, n.a.: not available; TC: tonic-clonic seizures, VPA: valproic acid.

Figure 1 2D and 3D-position of the variants

(A) Top-view and ***(B)*** side-view of α1β3γ2 $GABA_A$ receptor cryo-EM structure (pdb:6hup) [36] visualized using ChimeraX [37]. ***(C)*** 18 GABRA1 variants (yellow spheres) are mapped onto the GABAA receptor α1 subunit. Since the intracellular domain of the cryo-EM structure is unresolved, p.(Glu403Gln) variant cannot be mapped onto protein structure. ***(D)*** Canonical protein sequence of the $GABA_A$ receptor α1 subunit with key regions highlighted (signal peptide (pink), extracellular domain (blue) transmembrane helices (green) and loops (orange), and C-terminus (pale blue)) depicting the position and 19 variants.

Figure 2 Functional analysis of α1β3γ2 $GABA_A$ receptors containing *GABRA1* variants.

(A) cRNA for the wild-type receptor and receptors containing a single variant *GABRA1* subunit was injected into *Xenopus laevis* oocytes and subjected to two electrode voltage clamp experiments as described in the methods. ***(B)*** GABA concentration-response relationships (CRRs) were generated for wild-type receptors and receptors containing variant *GABRA1* subunits on each experimental day. Representative traces depict CRRs for the wild-type receptor and receptors containing the p.(Val270Ala) and p.(Ser299Asn) *GABRA1* variants. ***(C)*** Normalised GABA CRRs were plotted as a function of the GABA concentration and the Hill equation was fitted to each dataset by non-linear regression. Datapoints are presented as mean ± SEM for n = 12-14 independent experiments. Dotted lines indicated the concentrations that lead to half maximal activation (EC_{50}) for each receptor type. Arrows indicated whether the variant leads to an increased (GoF) or a decreased (LoF) GABA sensitivity. ***(D)*** The difference in GABA sensitivities between wild-type and 19 variant receptors was calculated from the logarithmic conversion of EC_{50} values (ΔpEC_{50}, where p = -Log). Final ΔpEC_{50} datasets for each variant contain data from n = 11-14 independent experiments performed at minimally two independent experimental days. Statistical analysis was performed as described in the Table 1 legend and **** indicates $P < 0.0001$.

Figure 3 Clinical features and genotype-phenotype correlations of *GABRA1* variants

(***Left***) Correlations between phenotype, genotype (variant type and position) and functional effect (GoF vs LoF) for 24 subjects, carrying 17 different *GABRA1* pathogenic variants. Abbreviations: DEE = developmental and epileptic encephalopathy, GEFs+ = genetic epilepsy with febrile seizures plus, GoF = gain of function, LoF = loss of function. (**Right**) Variant locations mapped on the *GABRA1* subunit of the cryo-EM structure (pdb:6hup)[36] visualized using ChimeraX. Grey, red and purple spheres indicate functionally neutral, LoF and GoF variants respectively.

Figure 4 EEG patterns in the different *GABRA1* subgroups

Extracellular domain missense variants with LoF effect p.(Gly251Asp). ***(A)*** Pat.12, 9 year old, with a FE/GEFs+ phenotype, with infantile onset focal epilepsy and subsequently generalised seizures, moderate ID and behavioural disturbances. The EEG background is well structured. We observed trains of spike and slow waves in the right occipito-posterior temporal region, with occasional diffuse spreading. ***(B)*** Transmembrane helix TM3 missense variants with LoF effect Pt.22 p.(Ile317Leu). Pat.22, 17-months-old, with developmental delay and refractory GTC seizures. The interictal EEG showed a background slowing and frequent trains of 16 Hz activity in the central regions, with maximum central in the midline. ***(C)*** Transmembrane loop TM1-TM2 missense variants with LoF effect p.(Asn275Lys). Pat.17, 5 year old, from a

family with a GEFs+ phenotype. The interictal EEG shows trains of spike and slow waves bilaterally in the parieto-occipital regions, with occasional diffuse spreading. ***(D)*** Transmenbrane loop TM2- TM3 with LoF effect p.(Pro305Leu). Pat.21, a 10-year-old girl with mild ID and childhood onset epilepsy with prolonged focal autonomic seizures. Intermittent photic stimulation performed induces photoparoxysmal response.***(E)*** Transmembrane helix TM4 missense variants with GoF effect p.(Tyr438Cys). Pat.24, 8 year old, with mild-to-moderate ID and drug resistant atypical absences, focal motor seizures and NCSE. The EEG shows generalized 2.5-3 Hz spike and slow waves. Interestingly, EEGs from subjects harbouring LoF variants in the extracellular compartment (A+C), that can be distinguished from the EEG of subjects with transmembrane variants (B+D). EEG parameters: band pass filter 0.5-70 Hz; notch off.

Supplementary Table Electro-clinical features of the *GABRA1* cohort

Abbreviations: aAb: atypical absences, Ab: absences, ADHD: attention deficit/hyperactivity disorder, ASD: autism spectrum disorder, ASM: antiseizure medications, At: atonic, BGS: back-ground slowing, BRV: brivaracetam, CLB: clobazam, CNZ: clonazepam, CT: centro-temporal, D: discharges, DD: developmental delay, DE: developmental encephalopathy, DEE: developmental and epileptic encephalopathy, DQ: development quotient, EEG: electroencephalogram, F: female, FDG PET: fluorodeoxyglucose positron emission tomography, FE: focal epilepsy, FS: febrile seizures, FT: fronto-temporal, fu: follow-up, G: generalized, GEFs+: genetic epilepsy with febrile seizures plus, GMFCS: Gross Motor Function Classification System, GoF: gain of function, GTC: generalised tonic clonic seizures, ID: intellectual disability, IED: interictal epileptiform discharges, IGE: idiopathic generalized epilepsy, IQ: intelligence quotient, IS: infantile spasms, L: left, LCS: lacosamide, LEV: levetiracetam, LMT: lamotrigine, LoF: loss-of-function, M: male, mo: months, MRI: magnetic resonance imaging, my: myoclonus, NA: not available, PB: phenobarbital, PNES: psychogenic non epileptic seizures, PO: parieto-occipital, PT: parieto-temporal, R: right, RUF: rufinamide, Sp: spike, SE: status epilepticus, STP: stiripentol, SVT: supraventricular tachycardia, SW: spike and wave, Sz: seizures, T: tonic, TC: tonic-clonic, TPM: topiramate, VPA: valproic acid, VUS: variant of uncertain significance, y: years, ZNS: zonisamide. *: already reported in literature, $^{+}$: familial variant, CADD model GRCH37 v1.6

Table 1 Fitted and recorded data for α1β3γ2 receptors containing *GABRA1* variants

α1 variant	ΔpEC_{50} ± SD	n	*P* (ΔpEC_{50})	$GABA_{max}$ (nA) ± SD	n	*P* ($GABA_{max}$)	Functional outcome	ACMG
WT	0.00 ± 0.14	83	NA	1.00 ± 0.38	189	NA	NA	NA
Glu63Lys	0.05 ± 0.11	12	0.995	0.94 ± 0.44	29	0.2411	No Change	Likely benign
Ser96Cys	-0.10 ± 0.16	12	0.5366	1.05 ± 0.36	24	0.7984	No Change	Likely benign
Arg112Gln	-0.39 ± 0.17	11	<0.0001	0.92 ± 0.41	33	0.2265	LOF	Pathogenic
Arg112Trp	-1.03 ± 0.23	11	<0.0001	0.88 ± 0.53	24	0.0822	LOF	Pathogenic
Arg147Gln	-0.69 ± 0.17	11	<0.0001	0.88 ± 0.37	23	0.2951	LOF	Likely pathogenic
Val162Met	-0.29 ± 0.12	11	<0.0001	0.93 ± 0.38	24	0.4803	LOF	Likely pathogenic
Ser213Thr	-0.43 ± 0.12	11	<0.0001	0.86 ± 0.40	30	0.0358	LOF	Pathogenic
Arg214His	-0.39 ± 0.14	12	<0.0001	0.86 ± 0.35	30	0.0741	LOF	Pathogenic
Gly251Asp	-0.64 ± 0.11	12	<0.0001	0.72 ± 0.29	30	0.0013	LOF	Pathogenic
Tyr252His	0.42 ± 0.14	12	<0.0001	0.89 ± 0.35	27	0.5171	GOF	Pathogenic
Val270Ala	-0.88 ± 0.15	14	<0.0001	0.42 ± 0.25	30	<0.0001	LOF/LOF	Pathogenic
Asn275Lys	-0.23 ± 0.14	12	<0.0001	1.12 ± 0.52	24	0.5229	LOF	Likely pathogenic
Thr295Ile	-0.52 ± 0.13	12	<0.0001	0.80 ± 0.43	32	0.0704	LOF	Pathogenic
Ser299Asn	0.50 ± 0.22	12	<0.0001	1.10 ± 0.59	29	0.8029	GOF	Pathogenic
Pro305Leu	-0.45 ± 0.13	12	<0.0001	0.83 ± 0.33	30	0.0821	LOF	Likely pathogenic
Ile317Leu	-0.48 ± 0.11	12	<0.0001	0.95 ± 0.39	30	0.5714	LOF	Pathogenic
Ala332Thr	0.30 ± 0.15	11	<0.0001	0.77 ± 0.37	29	0.031	GOF	Pathogenic
Glu403Gln	0.14 ± 0.14	11	0.1557	0.85 ± 0.25	24	0.6295	No Change	Likely benign
Tyr438Cys	0.39 ± 0.16	12	<0.0001	0.94 ± 0.46	26	0.7248	GOF	Pathogenic

Table 1 Fitted and recorded data for α1β3γ2 receptors containing *GABRA1* variants

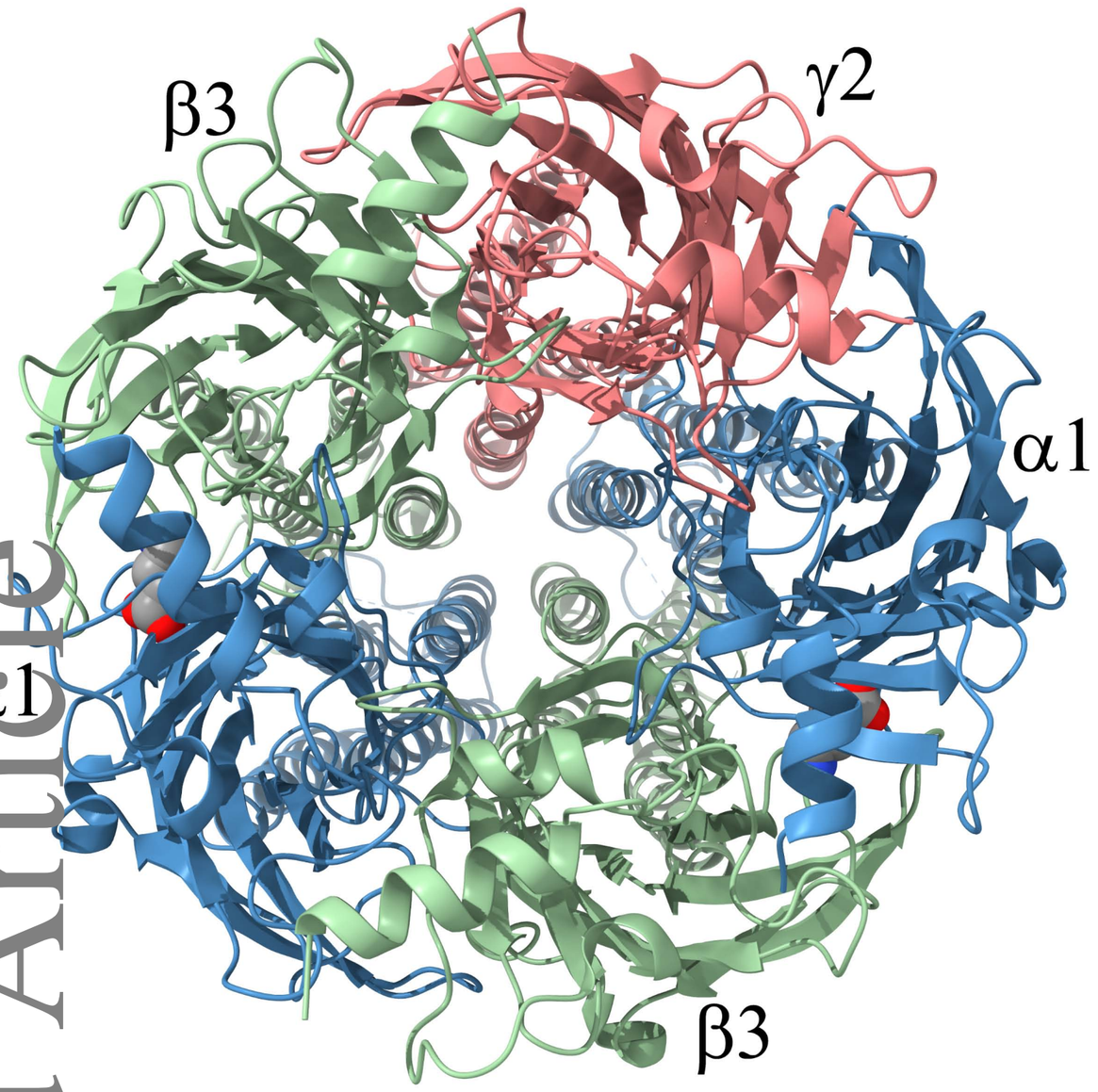
α1 variant	ΔpEC_{50} ± SD	n	*P* (ΔpEC_{50})	$GABA_{max}$ (nA) ± SD	n	*P* ($GABA_{max}$)	Functional outcome	ACMG
WT	0.00 ± 0.14	83	NA	1.00 ± 0.38	189	NA	NA	NA
Glu63Lys	0.05 ± 0.11	12	0.995	0.94 ± 0.44	29	0.2411	No Change	Likely benign
Ser96Cys	-0.10 ± 0.16	12	0.5366	1.05 ± 0.36	24	0.7984	No Change	Likely benign
Arg112Gln	-0.39 ± 0.17	11	<0.0001	0.92 ± 0.41	33	0.2265	LOF	Pathogenic
Arg112Trp	-1.03 ± 0.23	11	<0.0001	0.88 ± 0.53	24	0.0822	LOF	Pathogenic
Arg147Gln	-0.69 ± 0.17	11	<0.0001	0.88 ± 0.37	23	0.2951	LOF	Likely pathogenic
Val162Met	-0.29 ± 0.12	11	<0.0001	0.93 ± 0.38	24	0.4803	LOF	Likely pathogenic
Ser213Thr	-0.43 ± 0.12	11	<0.0001	0.86 ± 0.40	30	0.0358	LOF	Pathogenic
Arg214His	-0.39 ± 0.14	12	<0.0001	0.86 ± 0.35	30	0.0741	LOF	Pathogenic
Gly251Asp	-0.64 ± 0.11	12	<0.0001	0.72 ± 0.29	30	0.0013	LOF	Pathogenic
Tyr252His	0.42 ± 0.14	12	<0.0001	0.89 ± 0.35	27	0.5171	GOF	Pathogenic
Val270Ala	-0.88 ± 0.15	14	<0.0001	0.42 ± 0.25	30	<0.0001	LOF/LOF	Pathogenic
Asn275Lys	-0.23 ± 0.14	12	<0.0001	1.12 ± 0.52	24	0.5229	LOF	Likely pathogenic
Thr295Ile	-0.52 ± 0.13	12	<0.0001	0.80 ± 0.43	32	0.0704	LOF	Pathogenic
Ser299Asn	0.50 ± 0.22	12	<0.0001	1.10 ± 0.59	29	0.8029	GOF	Pathogenic
Pro305Leu	-0.45 ± 0.13	12	<0.0001	0.83 ± 0.33	30	0.0821	LOF	Likely pathogenic
Ile317Leu	-0.48 ± 0.11	12	<0.0001	0.95 ± 0.39	30	0.5714	LOF	Pathogenic
Ala332Thr	0.30 ± 0.15	11	<0.0001	0.77 ± 0.37	29	0.031	GOF	Pathogenic
Glu403Gln	0.14 ± 0.14	11	0.1557	0.85 ± 0.25	24	0.6295	No Change	Likely benign

Tyr438Cys	0.39 ± 0.16	12	<0.0001	0.94 ± 0.46	26	0.7248	GOF	Pathogenic

Table 2 Clinical characteristics of the *GABRA1* subgroups based on location and functional effects of variants.

	Missense variants				**Nonsense variant**
Domain	*N-terminus*	*Transmembrane domain*			
Position	Extracellular	Helices TM1, TM2, TM3	Helices TM1 TM2, TM3, TM4	Loops TM1-TM2 and TM2-TM3	N-terminal
Subjects / variants	11/7	3/3	4/4	4/2	2/1 *(1 family)*
Inheritance	8 *de novo,* 3 n.a.	2 *de novo,* 1 n.a.	4 *de novo*	*3 inherited,* 1 n.a.	*Maternal*
ID	Mild-mod 9/11 (82%) Severe 1/11 (9%) No 1/11 (9%)	Mild-mod 2/3 (67%) Severe 1/3 (33%)	Mild-mod 3/4 (75%) Severe 1/4 (25%)	Mild 4/4 (100%)	No (2/2) (100%)
Neurological examination	Normal 3/11 (27%) Poor coordination / mild ataxia 8/11 (73%)	Normal 1/3 (33%) Hypotonia 1/3 (33%) Hypotonic-dystonic cerebral palsy 1/3 (33%)	Normal 2/4 (50%) Ataxia 1/4 (25%) Pyramidal signs + dystonia 1/4 (25%)	Normal 4/4 (100%)	Normal 2/2 (100%)
Epilepsy	11/11	3/3	3/4	4/4	2/2
Age at seizure onset (median and range)	8 mo (3– 48 mo)	8 mo (3– 48 mo)	27 mo (5 mo-10 y)	21 mo (9 mo- 4 y)	14,5 y (14 -15 y)
Syndromes	GEFS+	DEE	DEE / DE	GEFS+	IGE
Seizure types at onset	Focal 6/11 (55%) FS 3/11 (27%) GTC 1/11 (9%)	Focal 2/3 (67%) GTC 1/3 (33%)	IS 1/3 (33%) My + aAb 1/3 (33%) GTC 1/3 (33%)	FS 3/4 (75%) Focal 1/4 (25%)	GTC (1) Focal to bilateral TC (1)
Seizure types at follow up	Focal 7/11 (64%) GTC + My 5/11 (46%)	My 2/3 (66%) Ab 1/3 (33%) GTC 1/3 (33%)	aAb 1/3 (33%) Focal 1/3 (33%) GTC 1/3 (33%)	GTC 2/4 (50%) Ab 1/4 (25%) My 1/4 (25%)	GTC 1/2 (50%) Focal to bilateral TC 1/2 (50%)
Seizure outcome	Sz free 6/11 (55%) Treatable 4/11 (36%) Refractory 1/11 (9%)	Treatable 1/3 (33%) Refractory 2/3 (67%)	Sz free 1/3 (33%) Treatable 2/3 (67%)	Sz free 3/4 (75%) Treatable 1/4 (25%)	Sz free 2/2 (100%)
Fever sensitivity	9/10 (90%)	1/3 (33%)	0/2	3/4 (75%)	0/2
Photosensitivity	0/11	2/3 (67%)	0/2	2/4 (50%)	0/2
Most effective ASM	LEV (5/6), VPA (3/4)	LEV (2/3),VPA (1/1)	ACTH (1/1), VPA (2/2)	LEV (4/4)	CBZ (1/1)
Additional features	Behav dist 2/11 (18%) ADHD 1/11 (0,9%) ASD 2/11 (18%)	Behav dist 1/3 (33%) ADHD 1/3 (33%)	Behav dist 1/3 (33%) ASD 2/3 (67%)	ASD 1/4 (25%)	-
Recurrent variants	p.Arg112Gln p.Gly251Asp				

A



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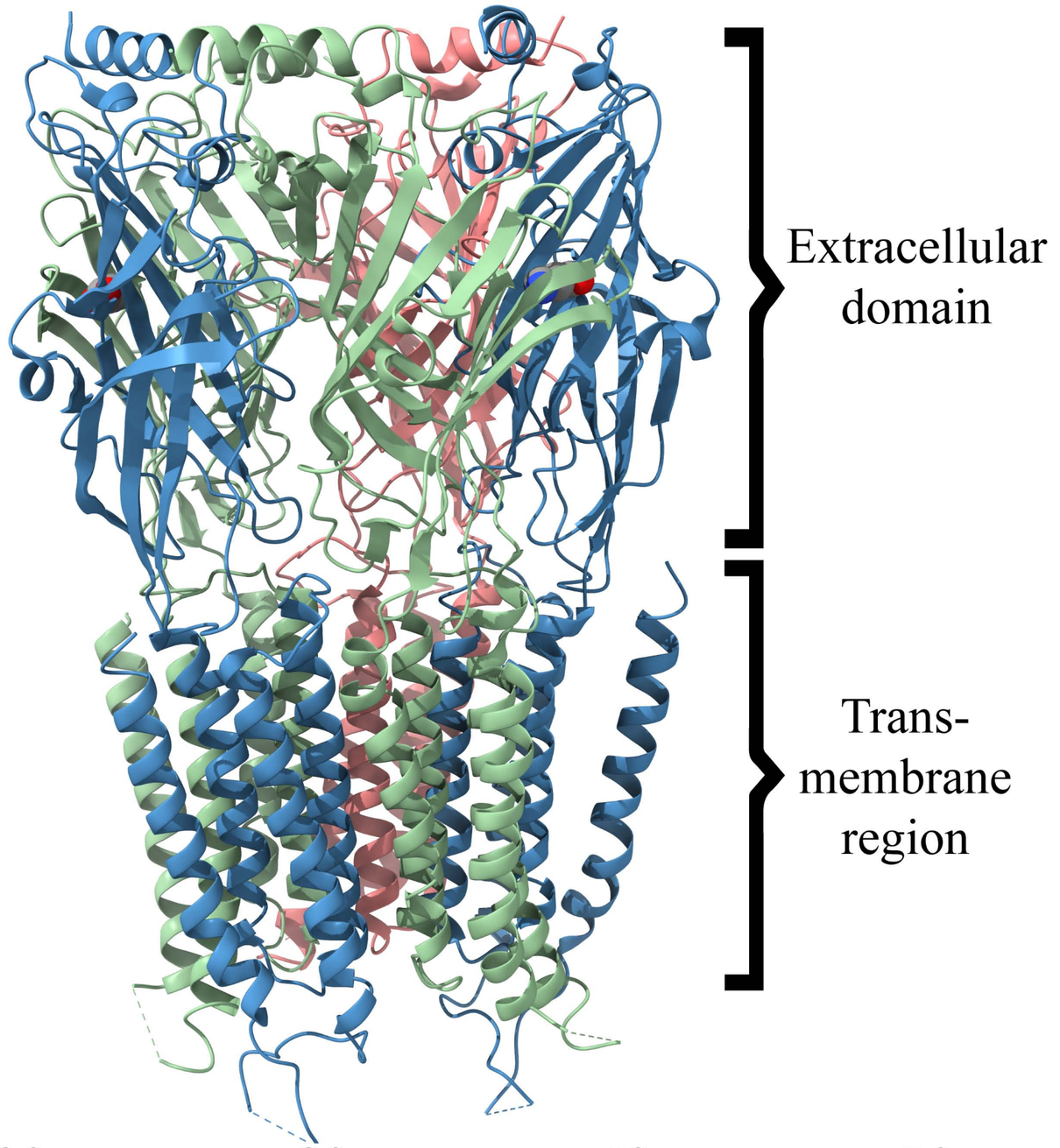

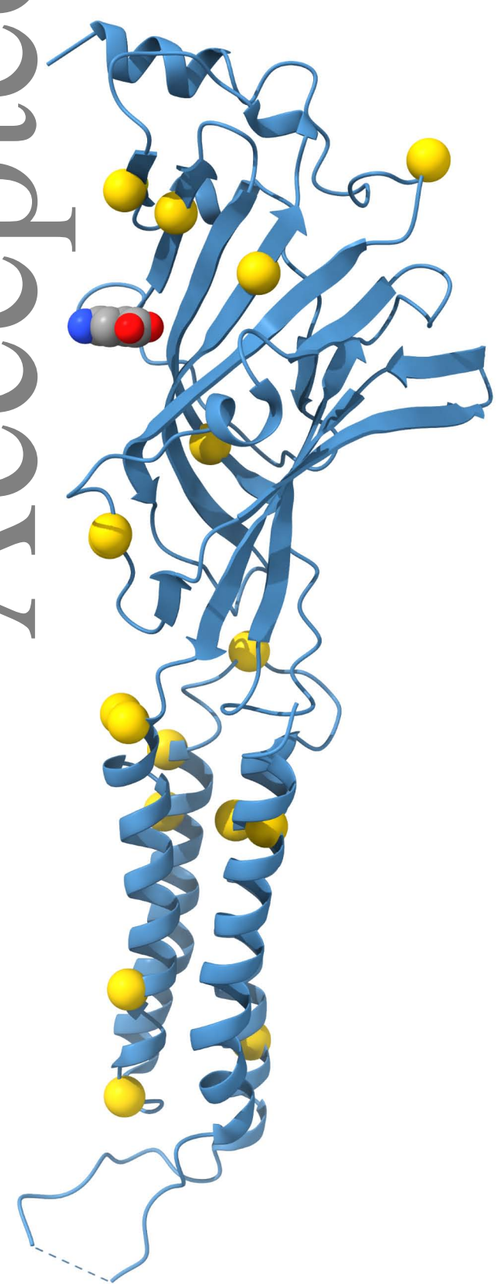

D

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MRKSPGLSDC LWAWILLLST LTGRSYGQPS LQDELKDNTT VFTRILDRLL

          60         70         80         90        100
DGYDNRLRPG LGERVTEVKT DIFVTSFGPV SDHDMEYTID VFFRQSWKDE
                 K                                   C
         110        120        130        140        150
RLKFKGPMTV LRLNNLMASK IWTPDTFFHN GKKSVAHNMT MPNKLLRITE
                 W                                   Q
         160   Q    170        180        190        200
DGTLLYTMRL TVRAECPMHL EDFPMDAHAC PLKFGSYAYT RAEVVYEWTR
                 M
         210        220        230        240        250
EPARSVVVAE DGSRLNQYDL LGQTVDSGIV QSSTGEYVVM TTHFHLKRKI
                   TH
         260        270        280        290        300
GYFVIQTYLP CIMTVILSQV SFWLNRESVP ARTVFGVTTV LTMTTLSISA
DH                       A       K                I   N
         310        320        330        340        350
RNSLPKVAYA TAMDWFIAVC YAFVFSALIE FATVNYFTKR GYAWDGKSVV
      L                  L                   T
         360        370        380        390        400
PEKPKKVKDP LIKKNNTYAP TATSYTPNLA RGDPGLATIA KSATIEPKEV

         410        420        430        440        450
KPETKPPEPK KTFNSVSKID RLSRIAFPLL FGIFNLVYWA TYLNREPQLK
   Q                                         C

APTPHQ
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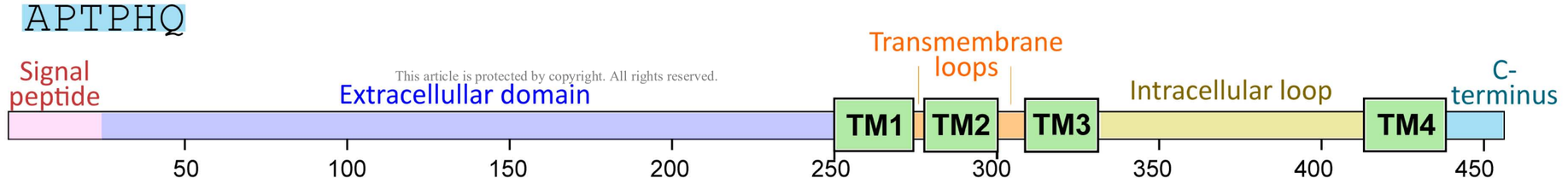

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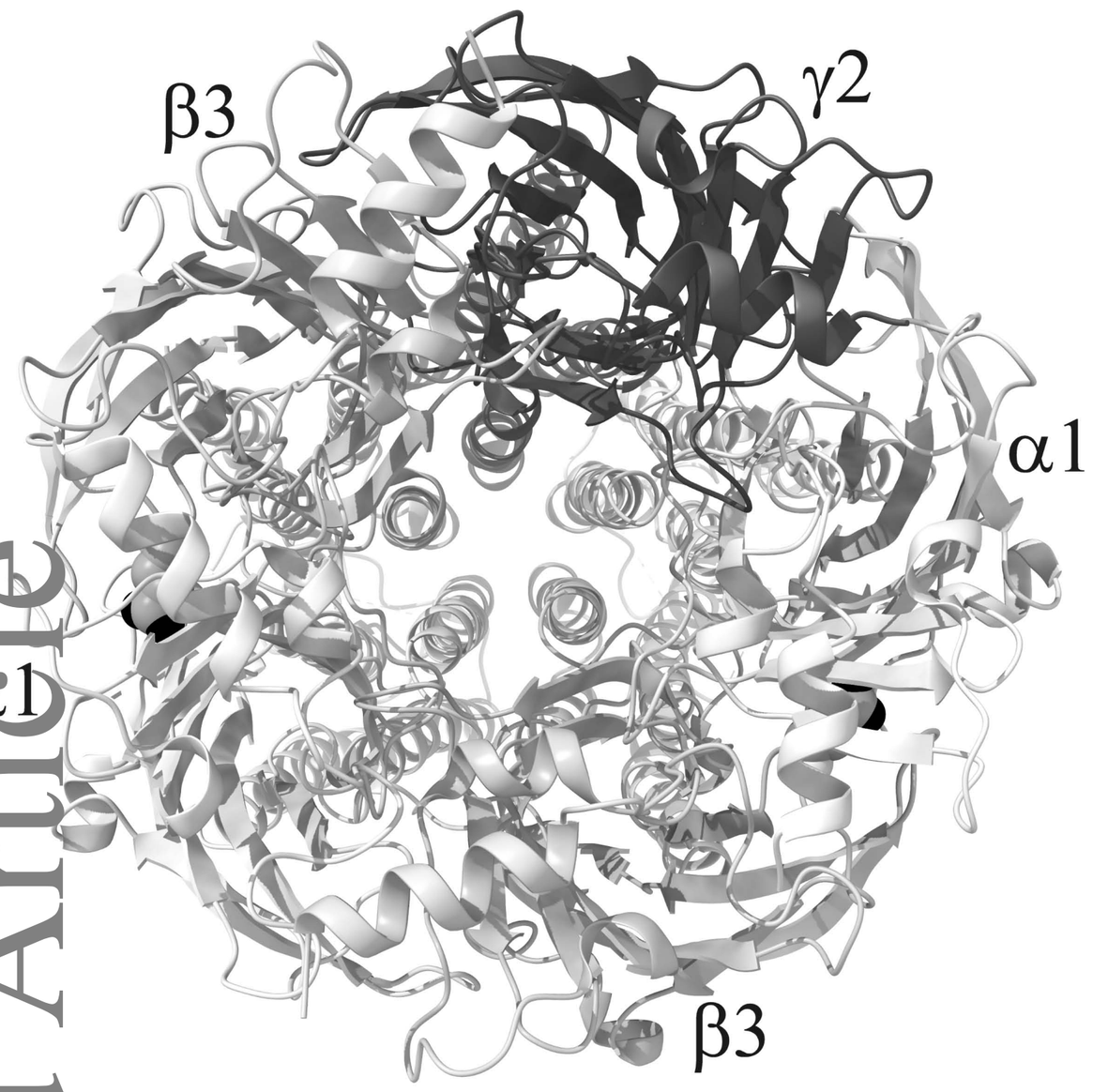

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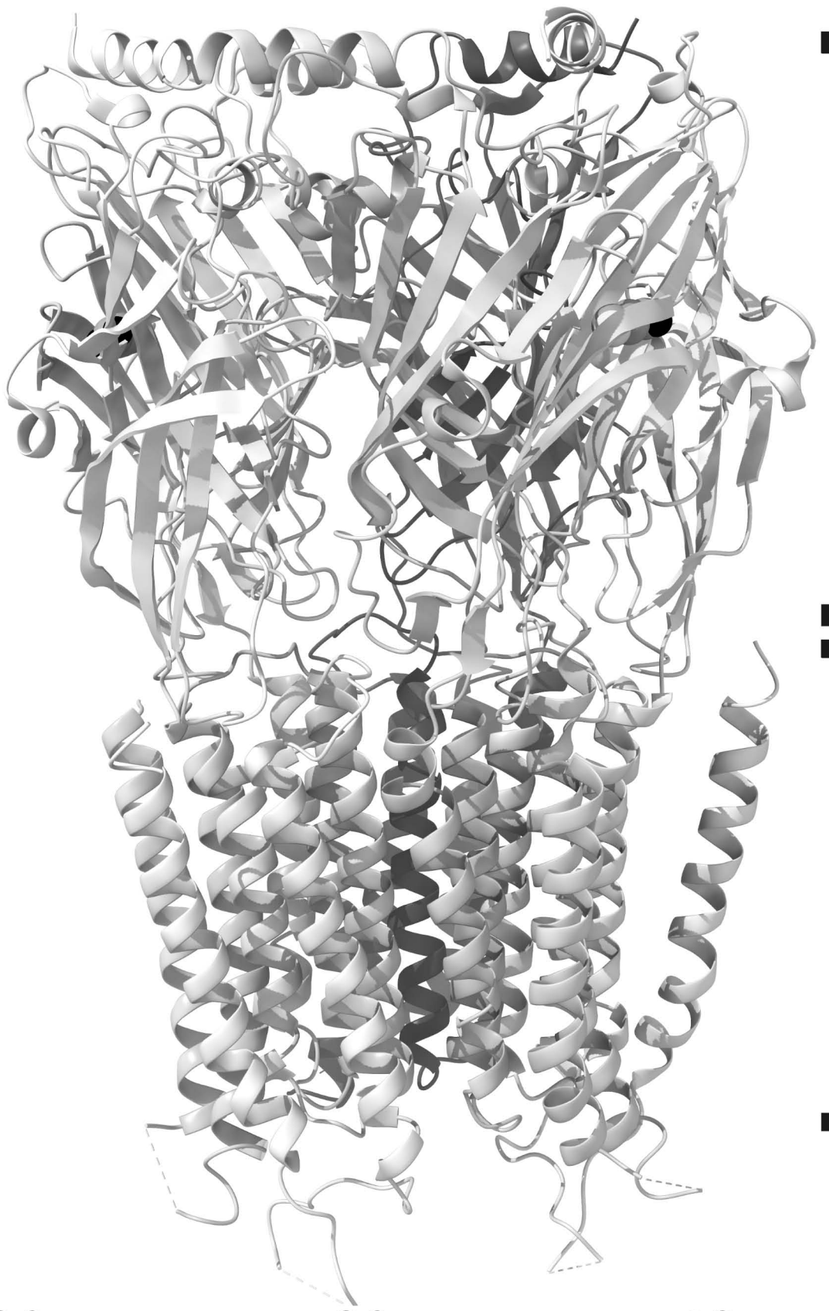

D

```
          10                  20                  30                  40                  50
MRKSPGLSDC LWAWILLLST LTGRSYGQPS LQDELKDNTT VFTRILDRLL

          60                  70                  80                  90                 100
DGYDNRLRPG LGERVTEVKT DIFVTSFGPV SDHDMEYTID VFFRQSWKDE
                 K                                   C
         110                 120                 130                 140                 150
RLKFKGPMTV LRRLNNLMASK IWTPDTFFHN GKKSVAHNMT MPNKLLRITE
                W                                    Q
         160   Q             170                 180                 190                 200
DGTLLYTMRL TVRAECPMHL EDFPMDAHAC PLKFGSYAYT RAEVVYEWTR
                M
         210                 220                 230                 240                 250
EPARSVVVAE DGSRLNQYDL LGQTVDSGIV QSSTGEYVVM TTHFHLKRKI
                   TH
         260                 270                 280                 290                 300
GYFVIQTYLP CIMTVILSQV SFWLNRESVP ARTVFGVTTV LTMTTLSISA
DH                       A       K                       I   N
         310                 320                 330                 340                 350
RNSLPKVAYA TAMDWFIAVC YAFVFSALIE FATVNYFTKR GYAWDGKSVV
     L                   L                       T
         360                 370                 380                 390                 400
PEKPKKVKDP LIKKNNTYAP TATSYTPNLA RGDPGLATIA KSATIEPKEV

         410                 420                 430                 440                 450
KPETKPPEPK KTFNSVSKID RLSRIAFPLL FGIFNLVYWA TYLNREPQLK
   Q                                                 C

APTPHQ
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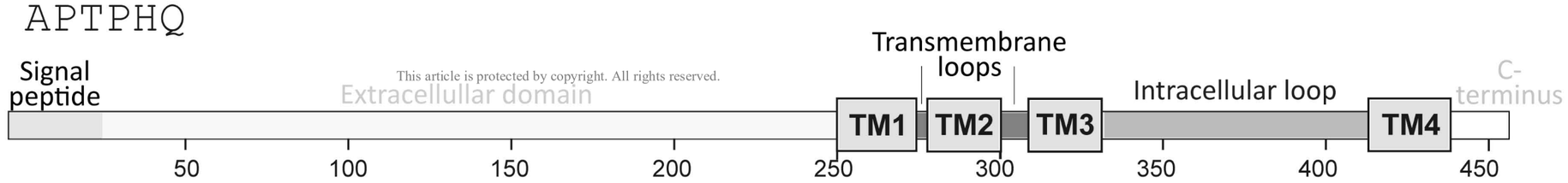
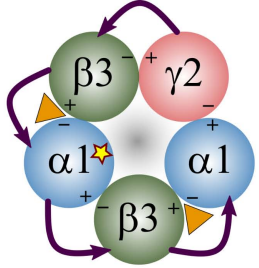


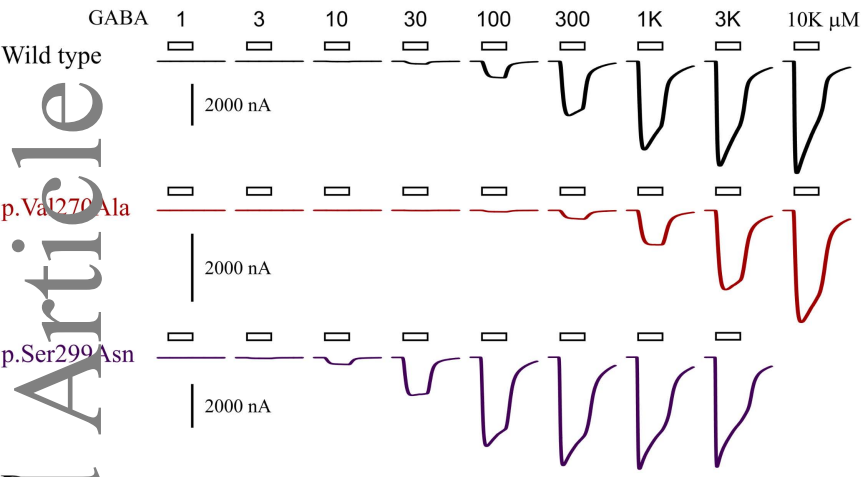
A

Concatenated cDNA construct design for expression of α1β3γ2 $GABA_A$ receptors that are heterozygous for *GABRA1* variants



B

Representative traces for wild type and *GABRA1* variant receptors



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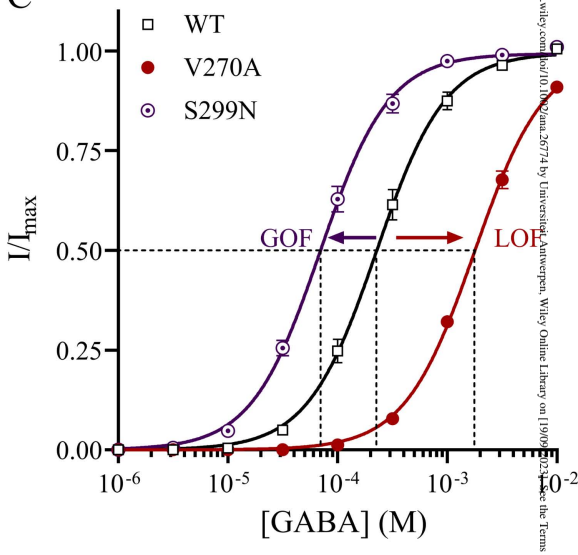
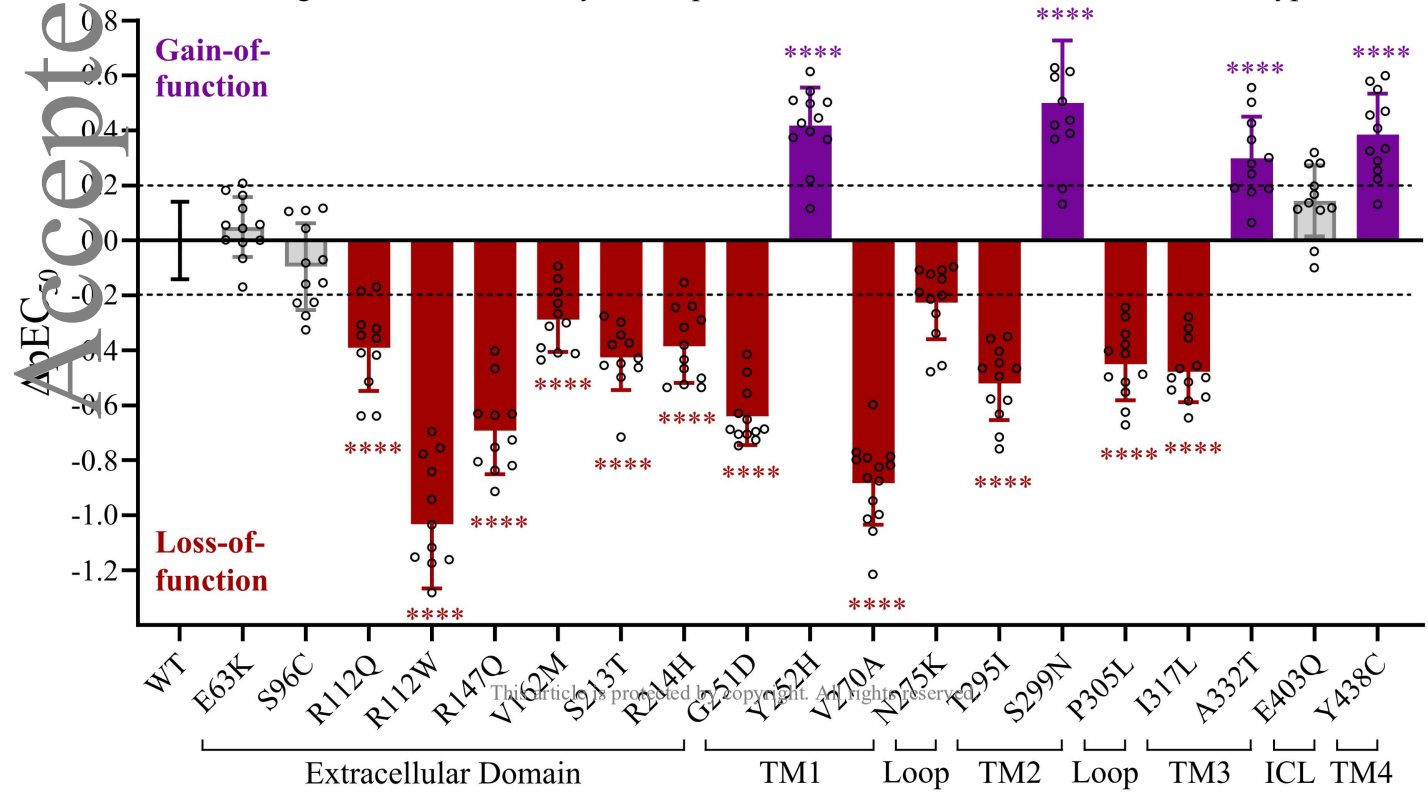
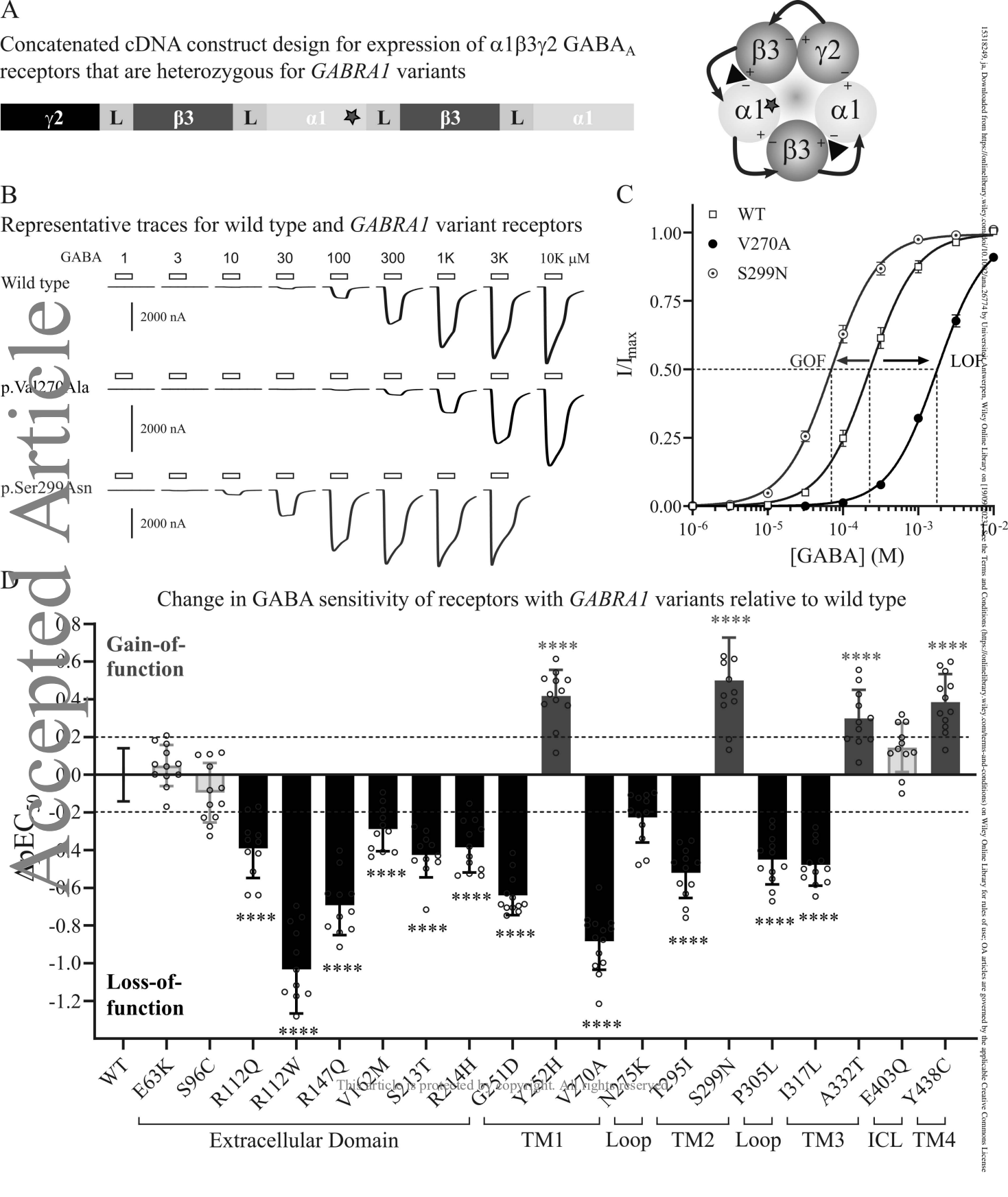


D

Change in GABA sensitivity of receptors with *GABRA1* variants relative to wild type





Clinical features and genotype-phenotype correlations of *GABRA1* variants

Nonsense variant

Late-onset epilepsy
- focal and GTC seizures
- good outcome
- normal development

LoF

Missense variants

TM1-TM2 loop — LoF — GEFs + Families

TM2-TM3 loop — LoF — Childhood onset focal epilepsy

Extracellular domain

Infantile or childhood onset epilepsy (homogenous phenotypes)

Infancy	Childhood	Late childhood
• focal hemiclonic seizures • fever sensitivity	focal autonomic seizures	possible myoclonic seizures

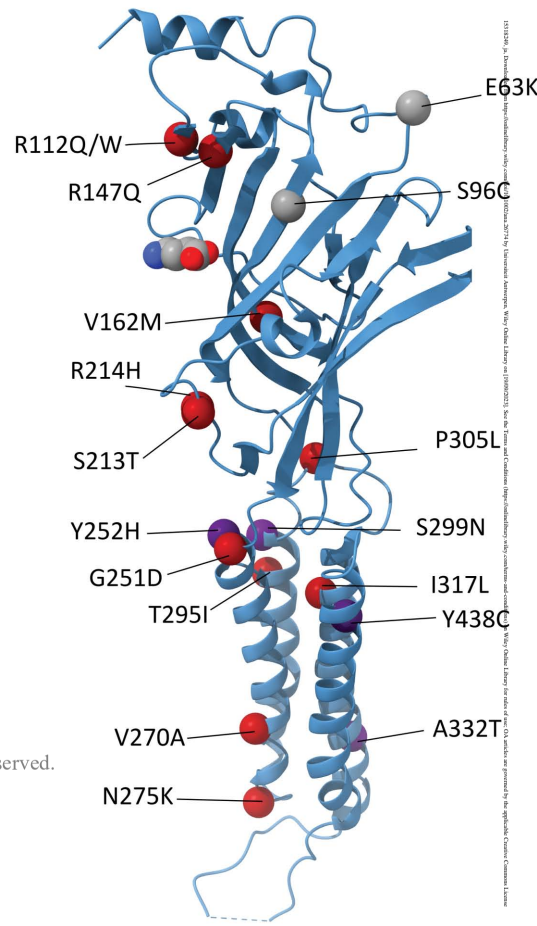
LoF

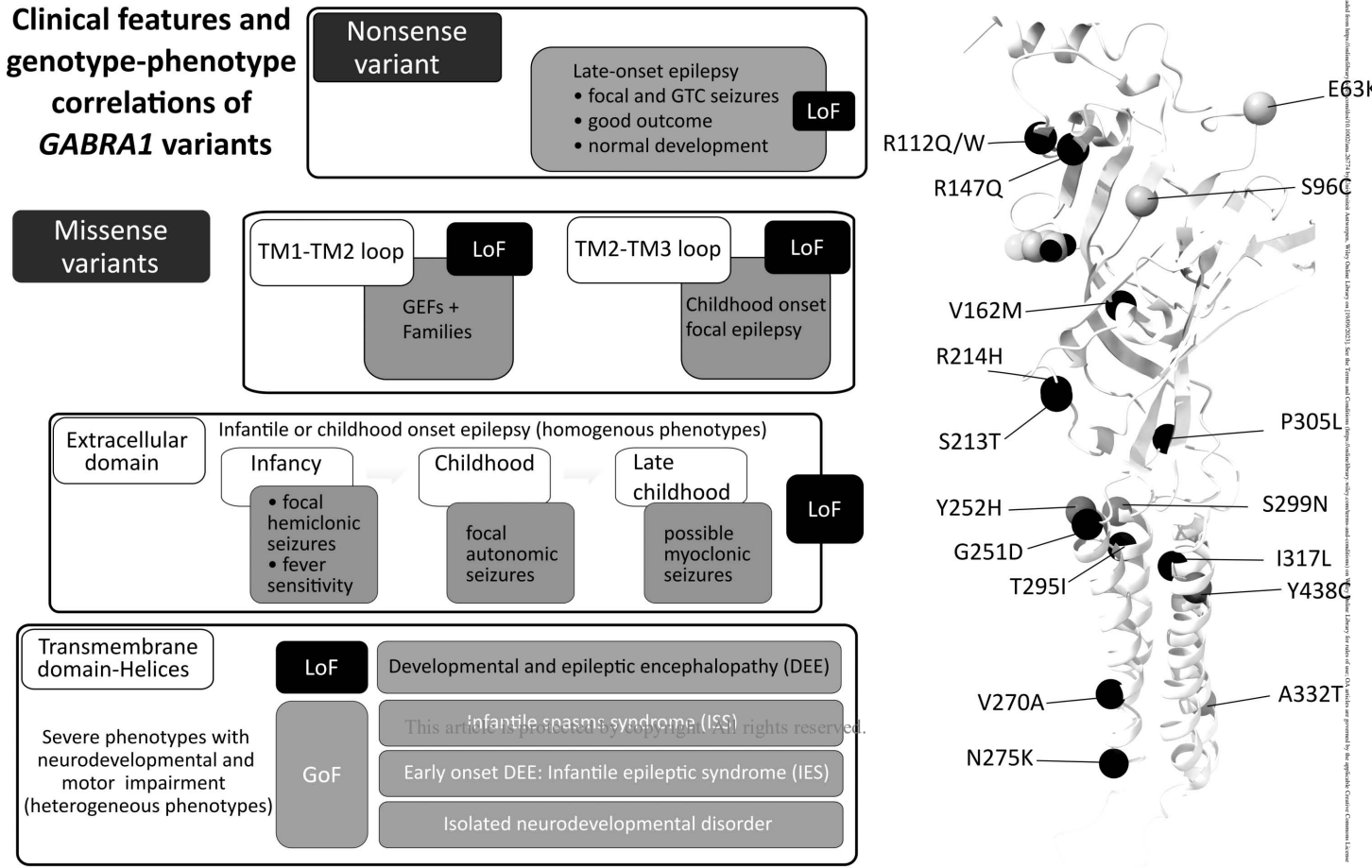
Transmembrane domain-Helices

Severe phenotypes with neurodevelopmental and motor impairment (heterogeneous phenotypes)

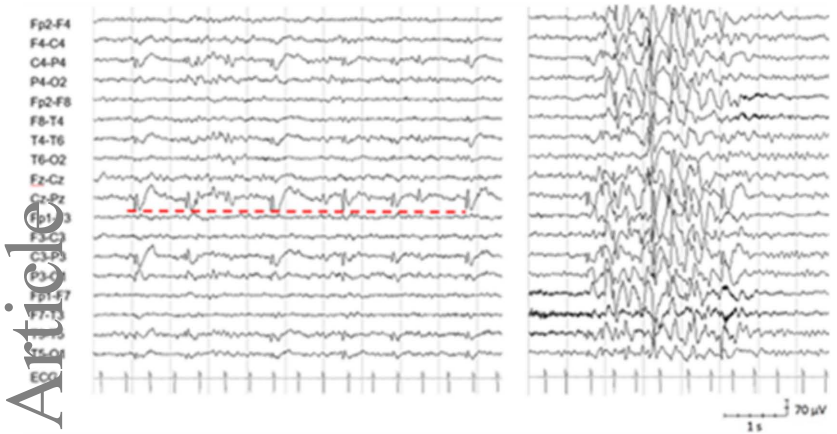
LoF
- Developmental and epileptic encephalopathy (DEE)

GoF
- Infantile spasms syndrome (ISS)
- Early onset DEE: Infantile epileptic syndrome (IES)
- Isolated neurodevelopmental disorder

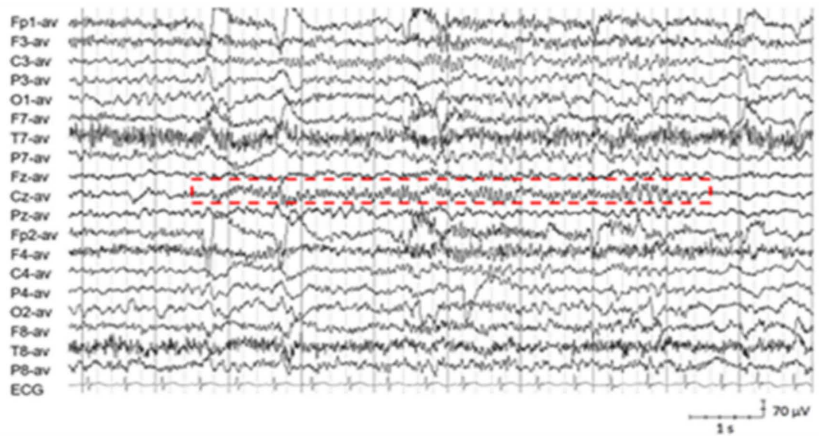




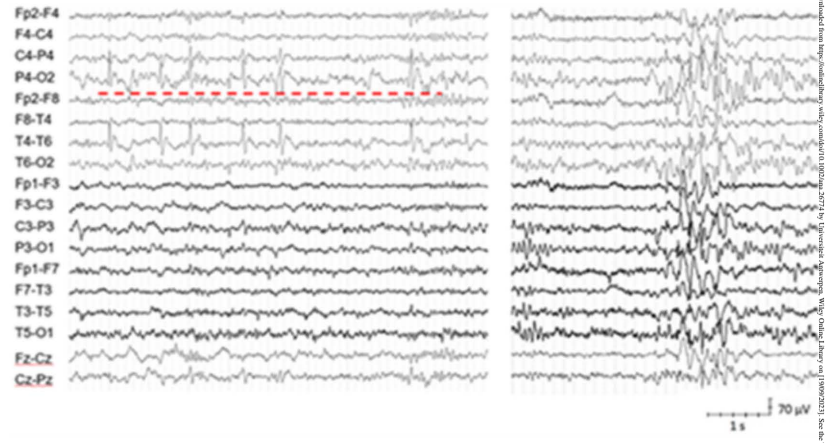
(A) Extracellular domain (LoF)



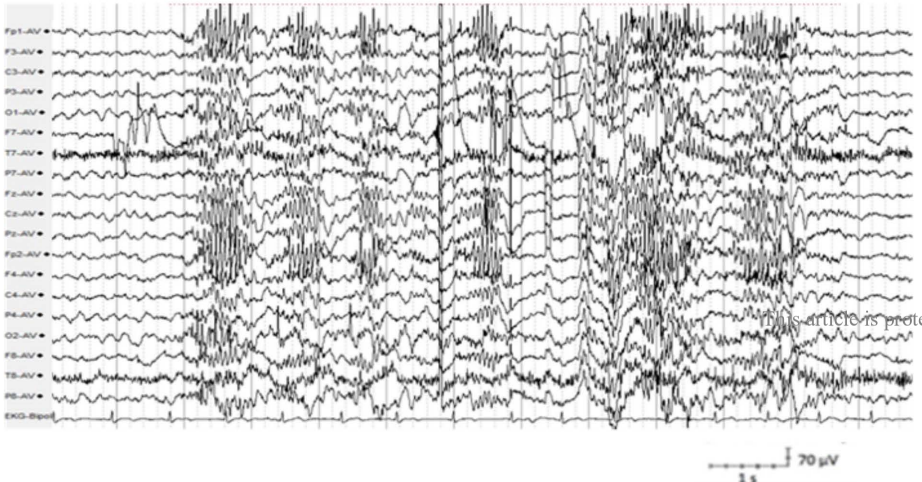
(B) Transmembrane domain – helicef (LoF)



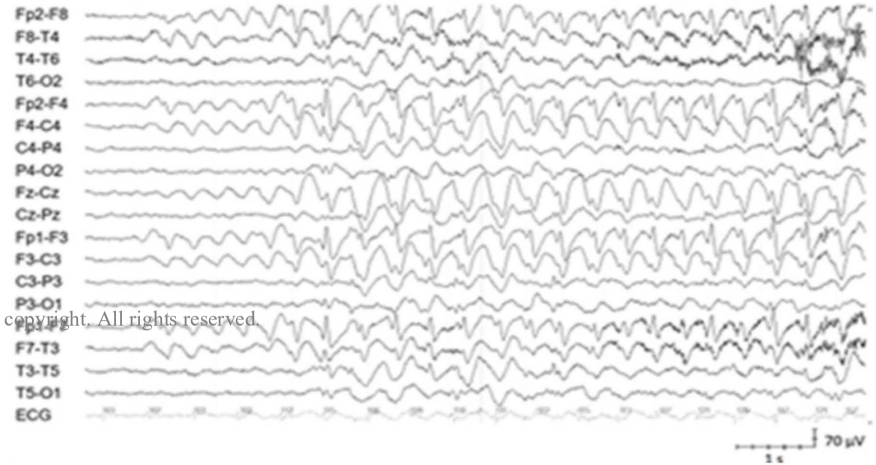
(C) Transmembrane domain – TM1-TM2 Loop (LoF)



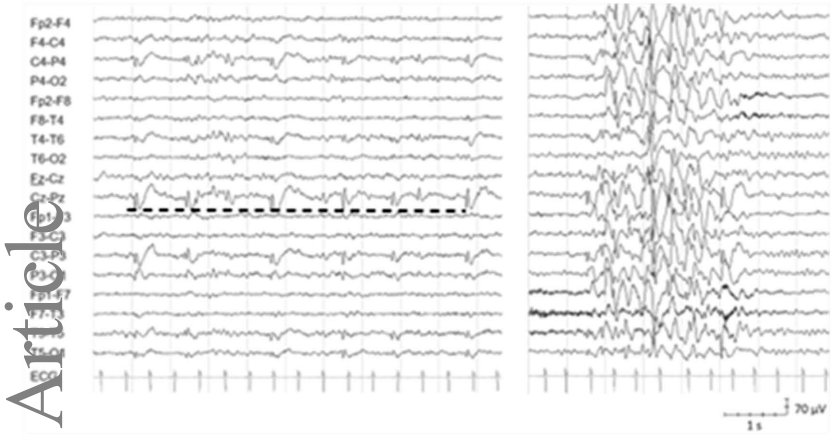
(D) Transmembrane domain TM2-T3 loop (LoF)



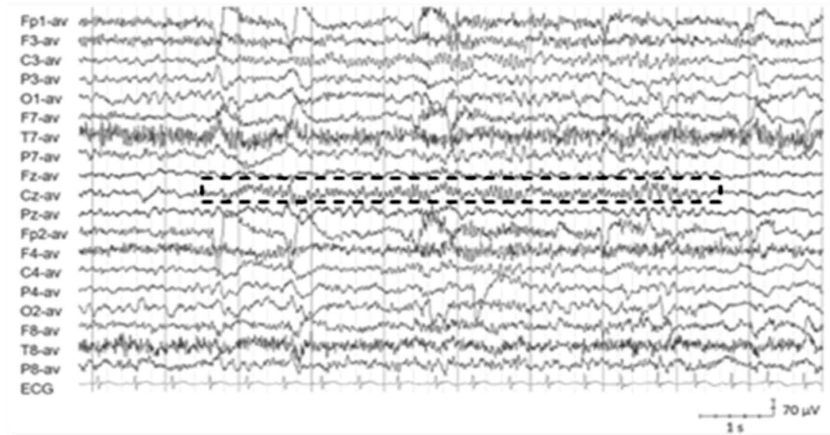
(E) Transmembrane domain – helices (GoF)



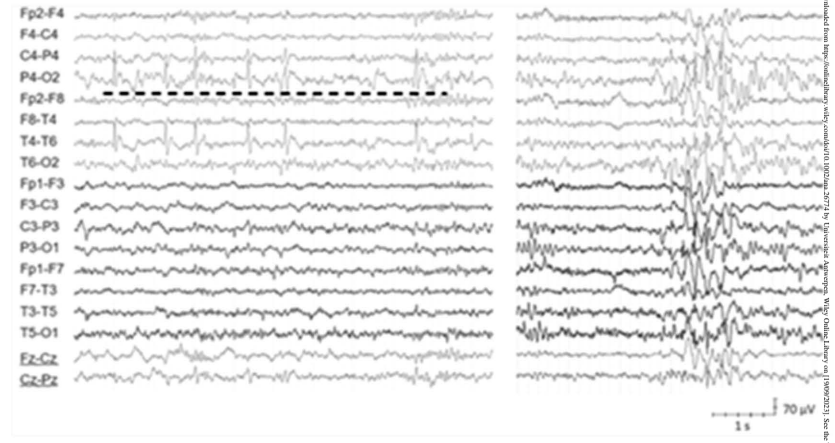
(A) Extracellular domain (LoF)



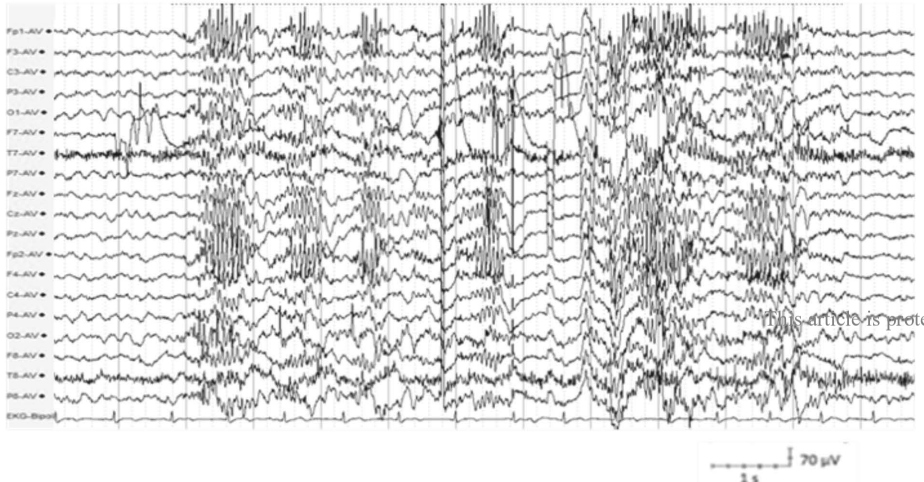
(B) Transmembrane domain – helicef (LoF)



(C) Transmembrane domain – TM1-TM2 Loop (LoF)



(D) Transmembrane domain TM2-T3 loop (LoF)



(E) Transmembrane domain – helices (GoF)