Open access • Journal Article • DOI:10.1212/WNL.0000000000003087

Phenotypic spectrum of GABRA1: From generalized epilepsies to severe epileptic encephalopathies — Source link

Katrine M Johannesen, Carla Marini, Siona Pfeffer, Rikke S. Møller ...+53 more authors

Institutions: University of Copenhagen, University of Florence, University of Southern Denmark, University of Tübingen ...+13 more institutions

Published on: 13 Sep 2016 - Neurology (Lippincott Williams & Wilkins)

Topics: Generalized epilepsy with febrile seizures plus, Epilepsy, Idiopathic generalized epilepsy, Juvenile myoclonic epilepsy and Seizure types

Phenotypic spectrum of *GABRA1*

From generalized epilepsies to severe epileptic encephalopathies

Katrine Johannesen, MD*
Carla Marini, MD, PhD*
Siona Pfeffer, MD*
Rikke S. Møller, MSc, PhD
Thomas Dorn, MD
Cristina Elena Niturad, PhD
Elena Gardella, MD, PhD
Yvonne Weber, MD
Marianne Søndergård, MD
Helle Hjalgrim, MD, PhD
Mariana Nikanorova, MD, PhD
Felicitas Becker, MD
Line H.G. Larsen, MSc
Hans A. Dahl, MSc, PhD
Oliver Maier, MD
Davide Mei, MD
Saskia Biskup, MD, PhD
Karl M. Klein, MD, PhD
Philipp S. Reif, MD
Felix Rosenow, MD
Abdallah F. Elias, MD
Cindy Hudson, MD
Katherine L. Helbig, MSc
Susanne Schubert-Bast, DrMed
Maria R. Scordo, MD
Dana Craiu, MD‡
Tania Djémié, PhD‡
Dorota Hoffman-Zacharska, MD‡
Hande Caglayan, PhD‡
Ingo Helbig, MD‡
Jose Serratosa, MD‡
Pasquale Striano, MD, PhD‡
Peter De Jonghe, MD, PhD‡
Sarah Weckhuysen, MD, PhD‡

Author list continued on next page

ABSTRACT

Objective: To delineate phenotypic heterogeneity, we describe the clinical features of a cohort of patients with *GABRA1* gene mutations.

Methods: Patients with *GABRA1* mutations were ascertained through an international collaboration. Clinical, EEG, and genetic data were collected. Functional analysis of 4 selected mutations was performed using the *Xenopus laevis* oocyte expression system.

Results: The study included 16 novel probands and 3 additional family members with a disease-causing mutation in the *GABRA1* gene. The phenotypic spectrum varied from unspecified epilepsy (1), juvenile myoclonic epilepsy (2), photosensitive idiopathic generalized epilepsy (1), and generalized epilepsy with febrile seizures plus (1) to severe epileptic encephalopathies (11). In the epileptic encephalopathy group, the patients had seizures beginning between the first day of life and 15 months, with a mean of 7 months. Predominant seizure types in all patients were tonic-clonic in 9 participants (56%) and myoclonic seizures in 5 (31%). EEG showed a generalized photoparoxysmal response in 6 patients (37%). Four selected mutations studied functionally revealed a loss of function, without a clear genotype–phenotype correlation.

Conclusions: *GABRA1* mutations make a significant contribution to the genetic etiology of both benign and severe epilepsy syndromes. Myoclonic and tonic-clonic seizures with pathologic response to photic stimulation are common and shared features in both mild and severe phenotypes. ***Neurology*® 2016;87:1140–1151**

GLOSSARY

cRNA = complementary RNA; **DS** = Dravet syndrome; **EE** = epileptic encephalopathy; **$GABA_A$** = γ-aminobutyric acid type A receptor; **GEFS+** = generalized epilepsy with febrile seizures plus; **GSW** = generalized spike-wave; **IGE** = idiopathic generalized epilepsy; **JME** = juvenile myoclonic epilepsy; **MAE** = myoclonic-astatic epilepsy; **WT** = wild type.

In the last 15 years, mutations in genes encoding the α_1, β_2, β_3, γ_2, or δ subunits (encoded by *GABRA1*, *GABRB2*, *GABRB3*, *GABRG2*, and *GABRD*, respectively) of the γ-aminobutyric acid type A ($GABA_A$) receptors have been associated with various forms of epilepsy, ranging from mild genetic generalized epilepsies and febrile seizures[1–5] (*GABRA1* and *GABRG2*) to severe epileptic encephalopathies (EEs) such as Dravet syndrome (DS), infantile spasms, and Lennox-Gastaut syndrome (*GABRA1*, *GABRB2*, and *GABRB3*).[6–8]

Mutations in the *GABRA1* gene were first identified in a large family with juvenile myoclonic epilepsy (JME)[9] and subsequently in a sporadic patient with childhood absence epilepsy who had a de novo frameshift mutation[4] (see the table). More recent studies have shown that mutations in *GABRA1* can also cause DS and other severe EEs.[6,7]

Functional studies indicate that mutations cause disease via haploinsufficiency and/or through a dominant negative effect on wild-type (WT) partnering subunits. The observed loss-of-function due to reduced protein stability and surface expression of $GABA_A$ receptors comprising mutant subunits, as well as decreased sensitivity to GABA, can lead to a subsequent reduction of inhibitory inputs in neurons and therefore increased excitability.[4,9,10]

*These authors contributed equally to this work.

‡Members of the EuroEPINOMICS-RES Dravet Working Group.

Authors' affiliations are listed at the end of the article.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Arvid Suls, MSc, PhD‡
Kai Muru, MD, PhD
Inga Talvik, MD
Tiina Talvik, DrMed‡
Hiltrud Muhle, MD
Ingo Borggraefe, MD
Imma Rost, MD
Renzo Guerrini, MD
Holger Lerche, MD
Johannes R. Lemke, MD*
Guido Rubboli, MD*
Snezana Maljevic, PhD*

Correspondence to
Prof. Dr. Lemke:
johannes.lemke@medizin.uni-leipzig.de
or Prof. Dr. Rubboli:
guru@filadelfia.dk

Supplemental data at Neurology.org

In this report, we describe the phenotypic spectrum observed in 19 individuals (16 probands and 3 additional family members) harboring different disease-causing mutations of the *GABRA1* gene.

METHODS *GABRA1* mutation–positive patients were identified from genetic screening in patients with different childhood epilepsies, mainly EEs. Patients were collected through an international collaboration that included clinical epilepsy and genetics centers in Europe and the United States. The probands and their families underwent detailed clinical examinations including personal interviews, review of the patients' charts, neuroimaging, and EEG investigations. Seizures were classified according to the International League Against Epilepsy Organization, and epilepsy syndromes were established when possible.[11] EEs were defined as a condition in which seizures and or epileptiform EEG abnormalities are believed to contribute to a progressive disturbance in cerebral function.[11] This concept is evolving and has not been fully established, which is why we classified some patients as mild EEs, a term not included in the current definition, to indicate that the clinical picture at onset was consistent with an EE yet the outcome was less severe than in other well-known EEs, such us Ohtahara syndrome or migrating malignant partial seizures of infancy.

Standard protocol approvals, registrations, and patient consents. Local ethical committees approved the study. All probands or, in case of minors, their parents or legal guardians, gave written informed consent before inclusion.

Mutation analysis. Genomic DNA was extracted from blood using standard methods. Mutations in 3 cases were identified using a targeted next-generation sequencing panel containing 85 epilepsy genes. This panel included all exons and at least 5 base pairs of flanking intronic sequences of *GABRA1* (RefSeq, hg19 build, transcript ID NM_000806.5) and was used to screen a cohort of 432 individuals with various forms of childhood-onset epilepsies including EEs. Variants were assumed to be pathogenic if they were nonsynonymous, splice-site altering, or frameshift changes, not present in 6,500 control samples (Exome Variant Server; see URLs/resources) or in the Exome Aggregation Consortium database consisting of exome data from 60,706 individuals (see URLs/resources), and had arisen de novo in the patient or were inherited from an affected parent. The remaining 13 *GABRA1* mutations were identified by clinical or research testing at 6 centers. Traditional Sanger sequencing was used to confirm all mutations and perform segregation analysis.

Mutagenesis and RNA preparation. The α_1 point mutations Ser76Arg, Arg214His, Lys306Thr, and Phe104Cys were introduced into the *GABRA1* complementary DNA inserted in the pcDNA3.1 vector (courtesy of Dr. P. Cossette), using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) and verified using Sanger sequencing. Primers are available on request. Complementary RNA (cRNA) of mutant and WT α_1, as well as β_2 and γ_{2short} subunits, was prepared using the T7 mMessage mMachine kit from Ambion (Austin, TX).

Oocyte preparation and injection. The use of animals and all experimental procedures were approved by local authorities (Regierungspräsidium Tübingen, Germany). Oocytes were obtained from the Institute of Physiology I, Tübingen, or purchased from EcoCyte Bioscience (Castrop-Rauxel, Germany) and prepared using established protocols (e-Methods at Neurology.org). cRNA concentrations were adjusted to 2 μg/μL and mixed in a 1:1:2 ratio ($\alpha_1/\beta_2/\gamma_{2short}$), or 0.5:0.5:1:2 for α_1(WT)/α_1(MUT)/β_2/γ_{2short} in the coexpression experiments. Seventy nanoliters of the corresponding cRNA mixtures was injected in the oocytes using Robooinject (Multi Channel Systems, Reutlingen, Germany).

Automated oocyte 2-microelectrode voltage clamp. GABA-evoked currents were recorded at room temperature (20°C–22°C) using Roboocyte2 (Multi Channel Systems). The intracellular glass microelectrodes had a resistance of 0.3–1 MΩ when filled with 1 M KCl/1.5 M KAc. The bath solution was ND96 (in mM: 93.5 NaCl, 2 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, and 5 HEPES, pH 7.5). The holding potential was −70 mV. Increasing GABA concentrations (in μM: 1, 3, 10, 30/40, 100, 300, and 1,000) were applied for 15 seconds, followed by a 3-minute washout period.

Data analysis. Recordings were analyzed using Roboocyte2+ (Multi Channel Systems), Clampfit (pClamp 8.2; Axon Instruments, Union City, CA), Microsoft Excel (Microsoft, Redmond, WA), Origin (OriginLab Corp., Northampton, MA), and Prism 6 software (GraphPad Software, La Jolla, CA) (e-Methods). Statistical differences were obtained using unpaired *t* test or Mann–Whitney test (Prism 6; GraphPad Software).

RESULTS **Mutation analysis.** Through an international collaboration including clinical epilepsy and genetic centers in Europe and the United States, we identified 16 patients with pathogenic heterozygous mutations in *GABRA1* including an additional 3 probands from multiplex families (figure 1). The phenotypic spectrum varied from JME, photosensitive idiopathic generalized epilepsy (IGE), and generalized epilepsy with febrile seizures plus (GEFS+) to myoclonic-astatic epilepsy (MAE) and severe EE. Segregation analysis was performed in 12 of 16 probands. Eleven mutations occurred de novo; the phenotypes of these patients were EE, MAE, and early infantile EE. Of the 4 cases with a positive family history, 3 were available for genetic testing, which showed maternal or paternal inheritance in concordance with the family history (figure 1). Familial cases were consistent with an autosomal dominant inheritance featuring additional family members with seizures and a phenotype concordant with the proband. Fourteen of the 16 mutations were missense whereas the other 2 disrupted a predicted splice-site. All mutations were in accord with the criteria mentioned above and were found at a highly conserved nucleotide across species (figure e-1); amino acid changes were predicted to be damaging by one or more of the prediction tools used (PolyPhen2 and SIFT; see URLs) (table). None of the mutations were present in the Exome Variant Server or the Exome Aggregation Consortium set of approximately 61,000 exomes (see URLs).

Phenotypic analysis. The table summarizes the clinical features of the 19 affected individuals (16 probands + 3 affected relatives) included in this study, as well as previously published cases. Age at inclusion ranged

Neurology 87 September 13, 2016

Table Clinical features of patients with *GABRA1* mutations

Pt ID	Sex, age[a]	Family history	Sz onset	Sz type	Sz type at follow-up	EEG	Development; MRI	Sz frequency/ outcome	Epilepsy/ syndrome	Mutation
1	F, deceased 37 y	Brother and maternal cousin with epilepsy	16 y	GTCS	GTCS	Bifrontal SW eye-closure sensitivity	Normal, depression, suicidal behavior; MRI normal	Not sz-free despite AEDs	JME	c.-248+1G>T; unknown inheritance
2[20]	NA	NA	NA	NA	NA	NA	NA	NA	IGE	c.59C>T; p.T20I; unknown inheritance
3	M, 18 mo	Father with febrile and afebrile sz	17 mo	Febrile and afebrile GTCS	Febrile and afebrile GTCS	Normal	Normal; MRI not performed	Sz-free on VPA	GEFS+	c.220G>A; p.V74I paternal
4	M, 16 y	Negative	5 mo	Hemiclonic	Hemiclonic, CSE, focal; secondary TC; GTCS, spontaneous or IPS-evoked eyelid My	Bilateral or multifocal PA	Normal at birth then severe delay, no language development, behavioral problems; MRI normal	AED-resistant daily sz	DS-like	c.226A>C; p.S76R; de novo
5	F, 8 y	Negative	6 mo	Asymmetrical GTCS, My, atypical Ab	Asymmetrical GTCS, My, atypical Ab	GSW, IPS response	Severe delay, no speech, autistic features; MRI normal	AED-resistant sz	EE	c.226A>C; p.S76R; de novo
6	a: M, 25 y (proband)	Yes (pts 5b, 5c)	13 y	My	My	GSW	Normal	Sz-free on VPA	JME	c.311T>G; p. F104C (maternal)
	b: M, 28 y (brother)	Yes (pts 5a, 5c)	7.5 y	TCS preceded by scotoma, headache, nausea	Rare TCS	GSW generalized IPS response	Normal	VPA + LTG, sz-free	IGE-GTCS	
	c: F, 48 y (mother)	Yes (pts 5a, 5b)	14 y	TCS preceded by scotoma	Rare TCS	GSW	Normal, migraine	Rare TCS until age 19 y then sz-free	IGE-GTCS	
7	F, 3.5 y	Paternal aunt had childhood-onset epilepsy and her son was diagnosed with Lennox-Gastaut syndrome	7 mo	Tonic-clonic, FS	Rare TCS	Normal	Normal gross motor development; speech and language delay (nonverbal); MRI: Chiari I malformation	Well controlled on LEV	DS-like	c.335G>A; p. R112Q; de novo
8[7]	F, 7 y	None	11 mo	Febrile, tonic-clonic sz	Absences, febrile dyscognitive, hemiclonic, myoclonic, tonic-clonic	Generalized SW	Moderate delay; MRI normal	NA	DS	c.335G>A; p. R112Q; de novo
9[7]	M, 18 y	NA	11 mo	Febrile, hemiclonic	Absences, atonic, febrile dyscognitive, hemiclonic, status epilepticus, tonic, tonic-clonic	Focal discharges	Moderate delay; MRI: calcified subependymal nodule in left lateral ventricle	NA	DS	c.335G>A; p. R112Q; de novo
10	F,1 y 10 mo	Negative	8 mo	Hypomotor	Clonic	Normal	Normal; MRI normal	Sz-free with LEV	Unclassified	c.335G>A; p. R112Q; de novo
11	F, 6 y	Negative	6 mo	Hemiclonic, febrile GTCS	Hemiclonic and secondary TC	CT, multifocal and bilateral spikes	Moderate delay, behavioral disorder; MRI normal	3–5 per wk	Mild EE	c.343G>A; p. N115D; de novo
12	M, 6.5 y	Negative	7 mo	Afebrile, prolonged hemiclonic	Afebrile, prolonged, hemiclonic, rare GTCS	Multifocal PA at onset then normal	Severe DD, no language	Sz every wk then monthly	DS-like	c.436C>A; p. L146M; de novo

Continued

Table Continued

Pt ID	Sex, age[a]	Family history	Sz onset	Sz type	Sz type at follow-up	EEG	Development; MRI	Sz frequency/ outcome	Epilepsy/ syndrome	Mutation
13	NA	NA	NA	NA	NA	NA	NA	NA	DS-like	c.641G>A; p. R214H; de novo
14	F, 35 y	Negative	15 mo	Prolonged FS	My, GTCS, atonic, focal	GSW, bifrontal PA	Normal at birth then severe delay; MRI normal	AED-resistant sz	EE	c.641G>A; p. R214H; de novo
15[20]	NA	NA	NA	Sz	Sz	NA	NA	NA	NA	c.643C>G; p. L215V
16[12]	F	Sister and sister's children with febrile sz and single GTC		Febrile sz, single GTC	Febrile sz, single GTC	Generalized spike-and-wave discharges	NA	NA	IGE	p.D219N; NA
17	M, 3 y	Negative	9 mo	Afebrile focal	hemiclonic	Slow background activity	Mild delay of speech development	Sz-free on VPA + LEV	Mild EE	c.752G>A; p. G251D; de novo
18[7]	F, 2 y		8 mo	Brief hemiclonic	Focal dyscognitive, hemiclonic, status epilepticus, tonic-clonic	Normal	Mild delay; MRI normal	NA	DS	c.751G>A; p. G251S; de novo
19[20]	NA	NA	NA	Abnormality of the nervous system	NA	NA	NA	NA	NA	c.788T>C; p. M263T
20	F, deceased at age 2 y	Negative	1st day of life	Clonic	Clonic, tonic asymmetrical, focal	BS, multifocal PA	Severe developmental delay from birth; MRI: severe delayed myelination	Daily sz, AED-resistant, died of pulmonary infection	EIEE	c.865A>C; p. T289P; de novo
21	F, 16 y	Negative	6 mo	Clonic, tonic, asymmetrical, focal	Focal and generalized clonic, tonic, myoclonic sz	Generalized activity	Moderate developmental delay; MRI normal	Once per mo	EIEE	c.865A>G; p. T289A; unknown
22[6]	NA	NA	NA	NA	NA	NA	NA	NA	DS	c.875C>T; T292I; de novo
23	M, 20 y	Negative	8 mo	MA	MA, My, TC	GSW, bifrontal SW, generalized IPS response	Developmental delay, behavioral problems; MRI at onset: delayed myelination; MRI at follow-up: normal	AED-resistant	MAE-like	c.917A>C; p. K306T; de novo
24[7]	M, 18 y	NA	8 mo	Hemiclonic, status epilepticus	Absences, atonic, febrile dyscognitive, hemiclonic, myoclonic, status epilepticus, tonic-clonic	Generalized SW, multifocal discharges, photoparoxysmal response	Mild delay; MRI normal	NA	DS	c.917A>C; p. K306T; de novo
25[9]	8 family members	JME in 4 generations	5–16 y	GTCS, myoclonus, absences	GTCS, myoclonus, absences	Generalized polyspike-and-wave discharges	NA	NA	JME	p.A322D; familial
26	a: M, 22 y (proband)	Positive (see 21b)	8 y	Afebrile TC	GTCS	Generalized IPS response	Normal; MRI normal	3 sz in total, sz-free on LEV	Photosensitive IGE	c.256-8T>G; maternal
	b: M, 18 y (brother)		8 y	Afebrile TC	GTCS	GSW and GPSW generalized IPS response, photoconvulsive reaction	Normal; MRI normal	3 sz in total, sz-free on LEV	Photosensitive IGE	

Continued

Table Continued

Pt ID	Sex, age[a]	Family history	Sz onset	Sz type	Sz type at follow-up	EEG	Development; MRI	Sz frequency/ outcome	Epilepsy/ syndrome	Mutation
27[5]	NA	Negative		NA	NA	NA	NA	NA	CAE	975delC; S326fs328X; de novo
28[12]	F, 2 cousins	Father of first cousin with unspecified epilepsy, mother of second cousin carrier of mutation		Febrile sz, single GTC	Febrile sz, single GTC	Generalized spike-and-wave discharges	NA	NA	IGE	p.K353delins18X; NA

Abbreviations: Ab = absences; AED = antiepileptic drug; BS = burst suppression; CAE = child absence epilepsy; CSE = convulsive status epilepticus; DD = developmental delay; DS = Dravet syndrome; EE = epileptic encephalopathy; EIEE = early infantile epileptic encephalopathy; FS = febrile seizures; GEFS+ = generalized epilepsy with febrile seizures plus; GPSW = generalized polyspike-waves; GSW = generalized spike-waves; GTC = generalized tonic-clonic; GTCS = generalized tonic-clonic seizures; ID = identification; IGE = idiopathic generalized epilepsy; IPS = intermittent photic stimulation; JME = juvenile myoclonic epilepsy; LEV = levetiracetam; LTG = lamotrigine; MA = myoclonic-astatic; MAE = myoclonic-astatic epilepsy; My = myoclonic; NA = not available; PA = paroxysmal activity; Pts = patients; SW = spike-wave; sz = seizures; TC = tonic-clonic; TCS = tonic-clonic seizures; VPA = valproic acid.

[a] At inclusion.

from 1.5 to 48 years, with a mean of 18 years. Two patients are deceased.

Analysis of electroclinical and neuroimaging data led us to classify 7 of the 19 patients as having IGE, specifically: JME (patients 1 and 6), IGE with generalized tonic-clonic seizures (2 relatives of patient 6), GEFS+ (patient 3), and photosensitive IGE (patient 26, and his brother). Of the remaining 12 patients, 3 had a more severe phenotype resembling MAE (MAE-like) (patient 23) and a mild, undefined EE (2 probands: 11 and 17) in whom early-onset seizures were associated with mild to moderate developmental delay and behavioral problems. One proband (10) experienced only a few seizures and is currently seizure free with normal development (at the age of 2). The last 8 probands were the most severely affected ones, with a phenotype resembling DS-like (4 probands: 4, 7, 12, and 13) and early-onset EE (4 probands: 5, 14, 20, and 21). These 8 individuals had intractable seizures, variably associated with severe developmental delay, behavioral problems, hand stereotypies, and autistic features (see the table and figure 2).

In the 11 patients of the EE group with available clinical information, seizure onset ranged from the first day of life to 15 months, with a mean of 7 months.

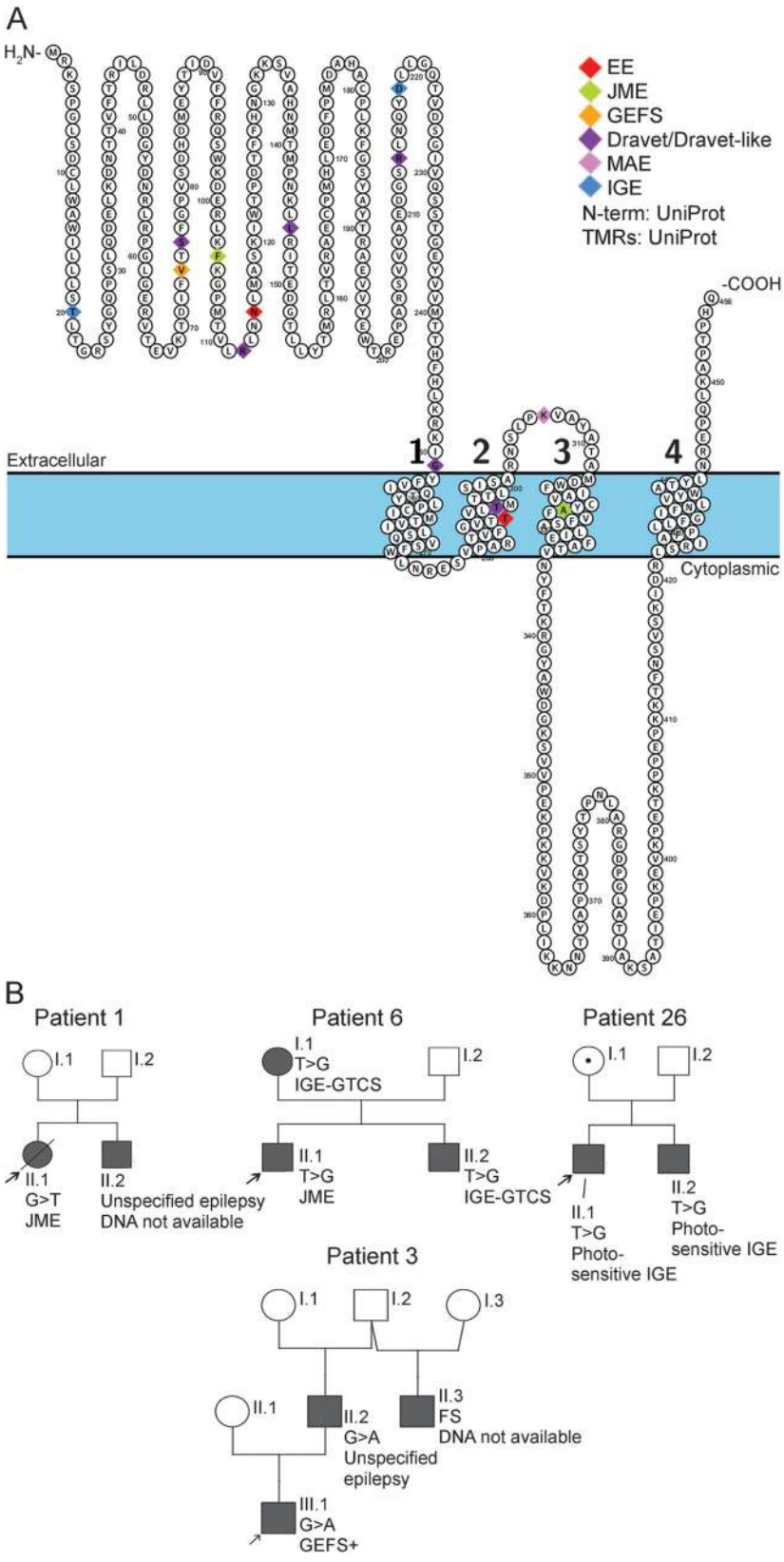
Seizure types included febrile seizures, focal, secondary generalized tonic-clonic, absences, tonic, atonic and myoclonic-atonic, and tonic-clonic seizures and convulsive status epilepticus. Myoclonic seizures, occurring in 6 patients, were the most common seizure type both in the benign and severe epilepsies. None of the patients had infantile spasms.

EEG recordings showed epileptiform discharges in 14 of 16 probands with available data, including generalized spike-wave (GSW) and generalized polyspike-wave complexes in 10 patients and a generalized photoparoxysmal response to intermittent photic stimulation (IPS) in 7 of the 16 patients (figure 3). In 2 patients, repeated EEGs could not capture epileptiform activity.

Seven of 15 probands with available information on treatment response were seizure free. Three patients with JME (1 and 6) and GEFS+ (3) were on valproic acid monotherapy, one patient with IGE (26), one patient (10) with unspecified epilepsy, and one patient with Dravet-like phenotype (7) were on levetiracetam monotherapy, and one patient with mild EE (17) was on valproic acid and levetiracetam combination therapy. Nine patients were drug-refractory after having tried multiple antiepileptic drugs in combination. Two patients were lost to follow-up. One patient with JME died of suspected suicide at the age of 37. The other patient, a girl with early infantile EE, died of pneumonia at the age of 2.

Functional analysis. Functional consequences of 4 selected mutations, associated with either severe

Figure 1 The *GABRA1* gene and pedigrees



(A) Mutations from both the literature and the present study are plotted in the gene, splice-site mutations excepted. (B) The pedigrees of the families with inherited mutations. EE = epileptic encephalopathy; GEFS = generalized epilepsy with febrile seizures; GTCS = generalized tonic-clonic seizures; IGE = idiopathic generalized epilepsy; JME = juvenile myoclonic epilepsy; MAE = myoclonic atonic epilepsy; TMRs = transmembrane regions.

Figure 2 Distribution of phenotypes

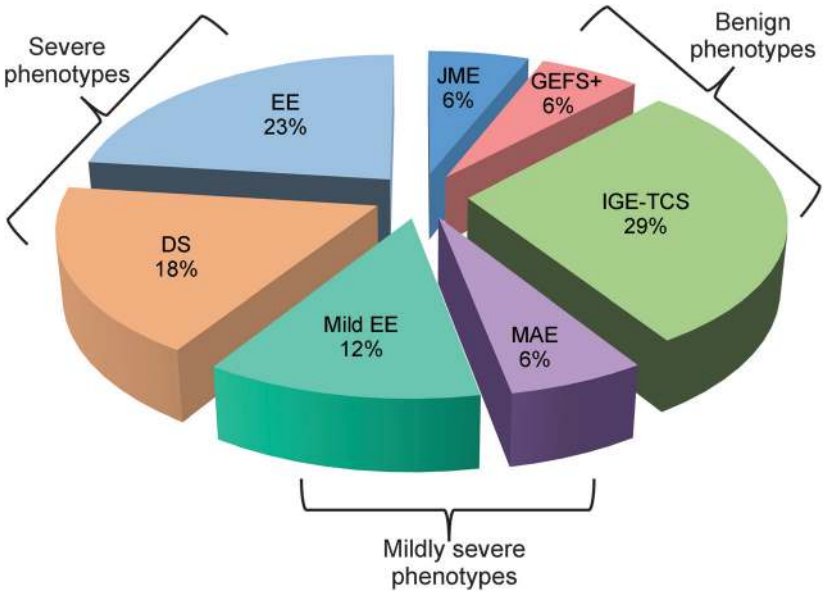
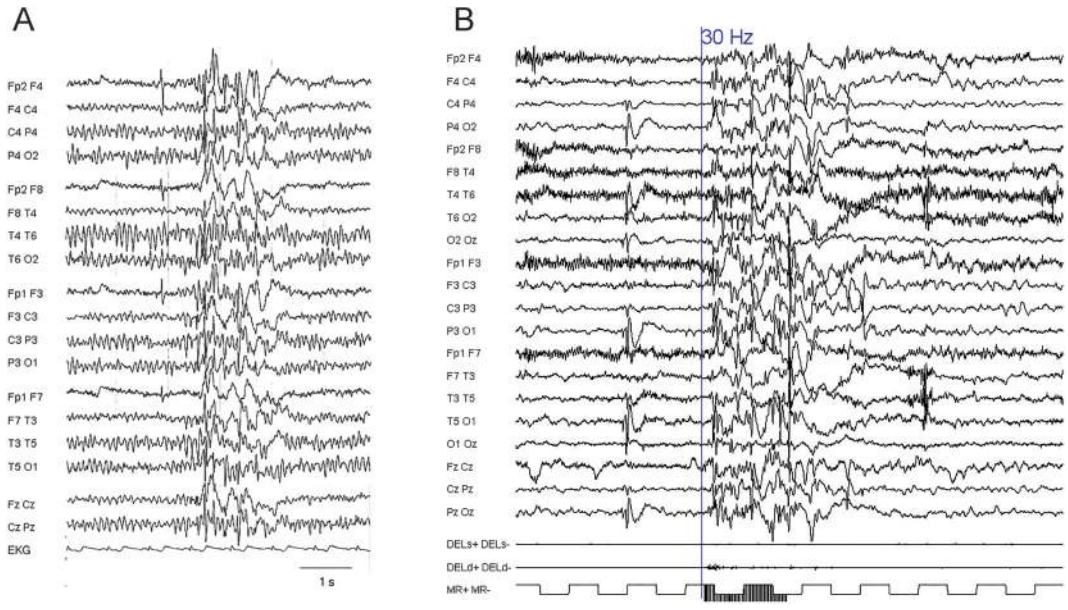


Diagram showing the distribution of the phenotypes throughout the gene. DS = Dravet syndrome; EE = epileptic encephalopathy; GEFS+ = generalized epilepsy with febrile seizures plus; IGE-TCS = idiopathic generalized epilepsy–tonic-clonic seizures; JME = juvenile myoclonic epilepsy; MAE = myoclonic-astatic epilepsy.

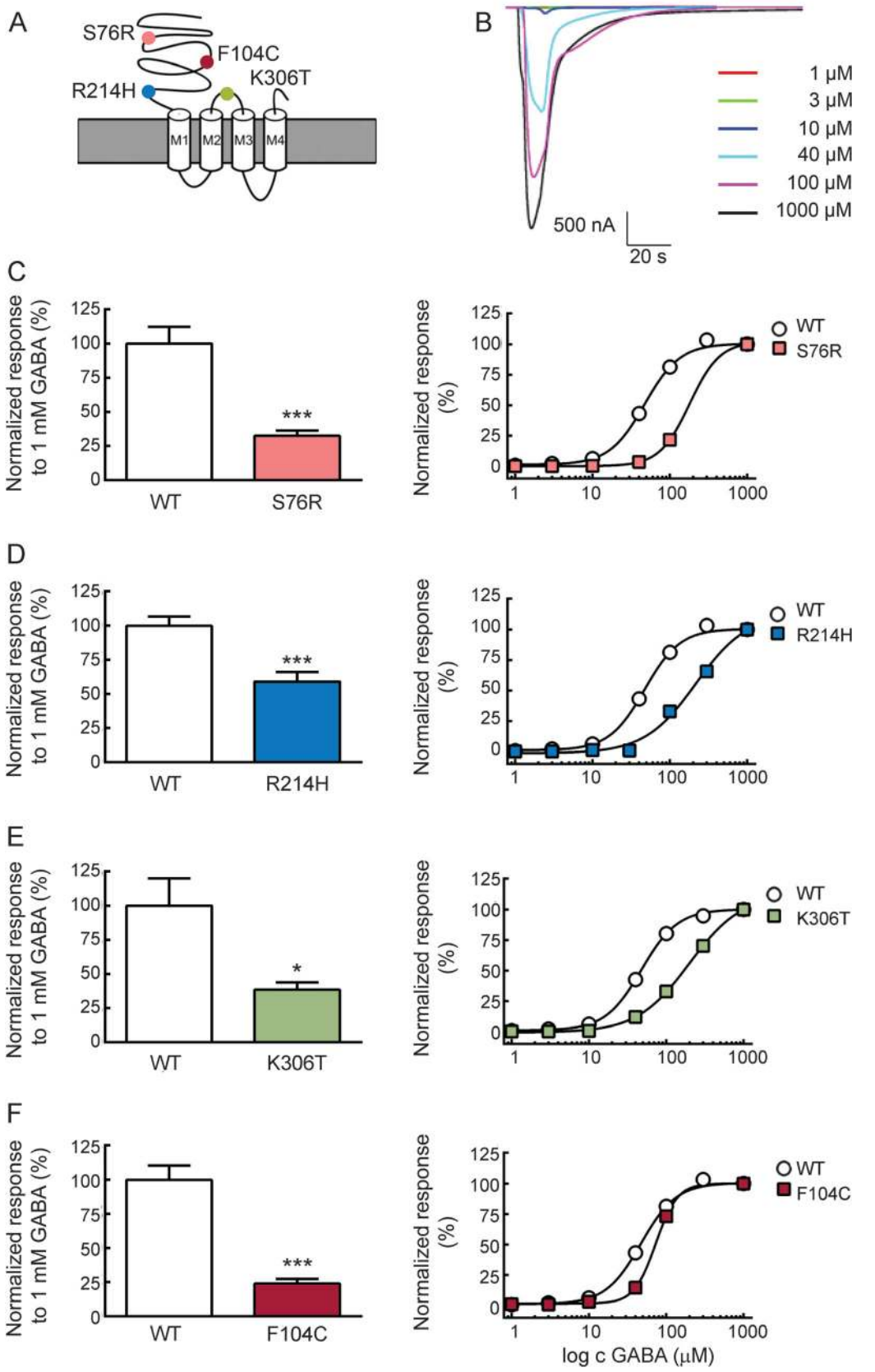
phenotype (Ser76Arg, Arg214His, and Lys306Thr) or IGE (Phe104Cys) were assessed using the *Xenopus laevis* oocyte expression system and automated 2-microelectrode voltage clamping. Three analyzed mutations are predicted to localize at the N-terminus and one in the extracellular M2-M3 loop of the $GABA_A$ receptor α_1 subunit (figure 4A). Coinjection with cRNA encoding β_2 and γ_{2s} subunits led to ionic currents, elicited by applying different concentrations of GABA (figure 4B). Current amplitudes evoked by a high GABA concentration (1 mM) revealed loss-of-function effects for all analyzed mutations (figure 4,

Figure 3 EEG from *GABRA1* patients



(A) Patient 1: Woman affected by juvenile myoclonic epilepsy. Interictal EEG showed focal spikes bilaterally in the frontal regions and brief irregular bursts of 2- to 2.5-Hz generalized spike-wave. (B) Patient 3: Boy with Dravet-like syndrome and an interictal EEG showing spontaneous focal spike and waves bilaterally in the posterior regions together with a generalized photoparoxysmal response during intermittent photic stimulation (IPS) (MR+ MR−: time scale and marker for IPS).

Figure 4 Functional analyses



Functional properties of selected *GABRA1* mutations Ser76Arg (S76R), Arg214His (R214H), Lys306Arg (K306T), and Phe104Cys (F104C) analyzed in *Xenopus laevis* oocytes. (A) Schematic representation of the α_1 subunit of the $GABA_A$ receptor, including the predicted positions of the 3 mutated amino acids at the N-terminus and in the M2-M3 linker. (B)

Continued

Figure 4 legend, continued:
Example of current responses to application of increasing concentrations of GABA (in μM: 1, 3, 10, 40, 100, and 1,000) recorded from *Xenopus* oocytes expressing $\alpha_1\beta_2\gamma_{2s}$ WT receptors. (C–F) Left: Current amplitudes of WT and mutant channels recorded on the same day in response to 1 mM GABA application were normalized to the mean value of the WT response for that day. Normalized current responses to 1 mM GABA application are shown in (C) for WT (n = 21) and S76R (n = 17), (D) for WT (n = 49) and R214H (n = 32), (E) for WT (n = 17) and K306T (n = 10), and (F) for WT (n = 25) and F104C (n = 18). Right: The dose-response curves for $\alpha_1\beta_2\gamma_{2s}$ WT and mutant receptors were obtained on application of different GABA concentrations, in μM: 1, 3, 10, 30 (R214H) or 40, 100, 300 (R214H, WT) and 1,000, and normalized to the maximal response (1,000 μM) for each cell. EC_{50} values were determined for each oocyte. The averaged EC_{50} values (in μM), shown as mean ± SEM, were: for the WT 49 ± 2 (n = 109), for S76R 174 ± 10 (n = 13, $p < 0.0001$), for R214H 222 ± 13 (n = 20, $p < 0.0001$), for K306T 200 ± 12 (n = 9, $p < 0.0001$), and for F104C 75 ± 4 (n = 18, $p < 0.0001$). Statistical significance: $*p < 0.05$, $***p < 0.0001$, using unpaired *t* test for the current amplitudes, or Mann–Whitney test for the EC_{50}. GABA = γ-aminobutyric acid; $GABA_A$ = GABA receptor type A. EC_{50} = half maximal effective concentration; WT = wild type.

C–F). This is the expected concentration of GABA in physiologic conditions, on its release into the synaptic cleft.[12] Currents recorded for Ser76Arg mutant subunits reached 32% ± 4%, for Arg214His 59% ± 7%, for Lys306Thr 39% ± 5%, and for Phe104Cys 24% ± 3% of those recorded for the WT on the same day (figure 4, C–F). We further coexpressed each of the mutants with the WT in a 1:1 ratio by substituting half of the amount of WT cRNA with the cRNA encoding mutant subunits, as expected in a heterozygous mutation carrier. The obtained currents were in the range of 60% to 80% of the WT, reaching significant difference only for the Ser67Arg coexpression (63% ± 9%, $p < 0.05$, data not shown), and thus, corresponding to the sum of the expected amplitudes for the WT and mutant channels, with no indication of a dominant negative effect. Decreased GABA sensitivity, revealed by a rightward shift of the dose-response curves, was found for all 4 mutations (figure 4, C–F). Hence, in *Xenopus laevis* oocytes, all analyzed mutated receptor subunits showed a clear loss-of-function compared to the WT.

DISCUSSION Our cohort shows a wide range of epilepsy subtypes spanning from the benign forms of unspecified epilepsy, JME, IGE with generalized tonic-clonic seizures, and GEFS+ to moderately severe phenotypes such as MAE and mild EE and at the end of the spectrum DS and other severe EEs. JME, a subtype of IGE was the first epilepsy phenotype linked to a *GABRA1* mutation more than a decade ago.[9] Since then, only 4 additional patients with IGE are on record[13,14] (see the table).

Modern technologies, including high-throughput and targeted next-generation sequencing using panels of genes and whole-exome sequencing, have increased the number of patients undergoing genetic analyses. This process has recently led to the identification of several novel *GABRA1* mutations with a more severe phenotype including DS and other less well-defined EEs, yet, only a few additional patients are on record[6,7] (table).

The current study increases the number of probands with *GABRA1* causative mutations and emphasizes the concept that they are associated with a large spectrum of phenotypes. Within the EE subgroup, some patients have a mild EE, whereas some have a severe early-onset epilepsy with severe developmental delay and EEG suppression burst pattern. The proportions observed in our study, 69% EE and 25% IGE, might suggest that *GABRA1* mutations more frequently manifest with a severe phenotype, although these figures are likely overestimated because of an ascertainment bias toward more severe probands.

Considering IGE phenotypes, we report an additional patient with JME after the initial description by Cossette et al.,[9] corroborating that *GABRA1* mutations are an uncommon cause of JME.

Detailed phenotypic analysis, especially regarding seizure types, suggests that tonic-clonic and myoclonic seizures are common clinical manifestations both in mild and severe *GABRA1*-related epilepsies. Similarly, EEG recordings show that GSWs are frequently observed in 90% of patients; 50% of patients have a generalized photoparoxysmal response during intermittent photic stimulation. Thus, tonic-clonic and myoclonic seizures with predominant GSWs and a photoparoxysmal response might represent shared and specific electroclinical features of *GABRA1* mutations and might be a hallmark of the underlying impaired functioning of GABA inhibition in the brain. The epilepsy severity and clinical heterogeneity could be explained by the genetic background or modifying genes also having a role in the epileptogenesis of other genetic epilepsy syndromes.

Similar to previously published studies, the majority of the identified *GABRA1* mutations are missense and arise de novo whereas only about one-third follows an autosomal dominant inheritance. Of note, there seems to be phenotypic concordance among the mutation carriers within the families, in contrast to the *SCN1A* mutations, which can cause different phenotypes within the same family.[15]

Five mutations were observed to be recurrent in this study and the literature: Ser76Arg, Arg112Gln, Arg214His, Gly251Ser/Asp, Thr289Pro/Arg, and Lys306Thr. Two patients (3 and 4) from this study had a de novo mutation at position Ser76Arg and shared

a similar phenotype with hemiclonic/hemiclonic-tonic seizures at onset, subsequent intractable myoclonic seizures, and severe developmental delay with autistic features. The Arg112Gln mutation detected in one of our patients (7) caused severe EE similar to previously published phenotypes (8[7]); however, it was also found in a girl presenting with only a few seizures and a normal psychomotor development (10). Two patients from this study (13 and 14) with EE and a Dravet-like phenotype harbored a de novo mutation, Arg214His. Furthermore, mutation Gly251Asp was detected in one of our patients with a mild EE phenotype (17), affecting the same position as in a previously reported patient with Gly251Ser exchange.[7] The difference in amino acid change could underline the slightly different phenotype. The same may be said about the Thr289Pro vs Thr289Arg, with the latter mutation leading to a milder phenotype. Finally, we found a de novo Lys306Thr mutation in a young man with an MAE-like phenotype (patient 23), sharing some clinical features with a previously published patient carrying the same mutation.[7] These isolated observations suggest some degree of genotype–phenotype correlation.

We selected 4 mutations for functional analysis, 3 recurrently occurring and found in patients with EE (Ser76Arg, Arg214His, Lys306Thr) and one from the family with JME (Phe104Cys). All of them caused a significant loss-of-function in *Xenopus laevis* oocytes. The coexpression with the WT yielded intermediate current responses underlining that in oocytes, the presence of mutant did not impair the function of the available WT subunits. Similarly to the reported JME-causing mutation,[4,9] the Phe104Cys change decreased current amplitudes and GABA sensitivity, and both effects were found for the 3 EE mutations. The dose-response shift for the Phe104Cys was observed only at GABA concentrations lower than 100 μM, whereas the dose-response curves for Ser76-Arg, Arg214His, and Lys306Thr indicated substantially reduced response to GABA in the whole range of tested concentrations. Thus, a combination of the diminished current amplitude and a dramatic loss of GABA sensitivity may present a functional hallmark of EE-associated *GABRA1* mutations.

The majority of *GABRA1* missense mutations identified thus far (12/18) localize at the N-terminus of the α_1 subunit, which contains the putative signaling peptide and is responsible for binding GABA and its agonists.[16] Other affected regions are the transmembrane segments M1 (1/18), the pore-forming M2 (3/18), and M3 (1/18) as well as the extracellular M2-M3 linker (1/18) involved in the coupling of agonist binding with gating.[17] Thus, the described *GABRA1* mutations affect regions critical for the proper function of $GABA_A$ receptors. Even though the oocyte expression system enabled us to analyze the biophysical properties of a pure population of heterologously expressed receptor proteins, we are aware of its limitations in determining the exact molecular mechanisms and thereby the pathophysiologic consequences of mutated receptors in neurons, neuronal networks, and the brain. However, this first functional screening tool has corroborated the presumed effect of the detected mutations in these patients.

The impaired inhibitory function of mutated $GABA_A$ receptors is expected to increase the excitatory activity and this disinhibition mechanism can lead to generation of seizures. Accordingly, a knockout *Gabra1* mouse model showed shorter viability and absence seizures in certain strain and sex backgrounds.[18] Furthermore, increased anxiety behavior was observed in these models,[19] which might be correlated to some comorbidities found in our patients.

The present study confirms *GABRA1* mutations as significant contributors to the genetic etiology of both mild and severe epilepsy syndromes. *GABRA1* mutations, through a possible mechanism of haploinsufficiency, cause an impairment of the GABA inhibitory function leading to a wide spectrum of epilepsy phenotypes. JME and other IGE phenotypes represent a minority of cases, with the majority of patients exhibiting infantile-onset severe epilepsies with associated cognitive and behavioral deficits. In some patients, the epilepsy phenotype, including age at onset, seizure types, and fever sensitivity, suggests a diagnosis of DS. Some previously published patients[7] and some of those included in this study have indeed been identified from a cohort of *SCN1A*-negative patients with DS.

Mutations in the *GABRA1* gene should be considered in patients with EE, especially those with infantile-onset, prominent tonic-clonic and myoclonic seizures, and GSWs and a photoparoxysmal response on the EEG. Genetic background and modifying genes might contribute to phenotypic heterogeneity.

AUTHOR AFFILIATIONS

From the Danish Epilepsy Center Filadelfia (K.J., R.S.M., E.G., M.S., H.H., M.N., G.R.), Dianalund, Denmark; Pediatric Neurology Unit (C.M., M.R.S., R.G.), Children's Hospital A. Meyer–University of Florence, Italy; Department of Neurology and Epileptology (S.P., C.N., Y.W., F.B., H.L., S.M.), Hertie Institute for Clinical Brain Research, University of Tübingen, Germany; Swiss Epilepsy Center (T. Dorn), Zurich, Switzerland; Institute for Regional Health Research (R.S.M., E.G., H.H., M.N.), University of Southern Denmark, Odense; Danish Epilepsy Center (G.R.), Filadelfia/University of Copenhagen, Dianalund; Amplexa Genetics (L.H.G.L.), Odense, Denmark; Children's Hospital (O.M.), St. Gallen, Switzerland; Department of Neuroscience (D.M.), Catholic University, Rome, Italy; CeGaT GmbH (S.B.), Tübingen; Epilepsy Center Frankfurt Rhine-Main (K.M.K., P.S.R., F.R.), Department of Neurology, Center of Neurology and Neurosurgery, University Hospital, Goethe University Frankfurt; Epilepsy Center Hessen (K.M.K., P.S.R., F.R.), Department of Neurology, University Hospitals Giessen and Marburg, Germany and Philipps University Marburg, Germany; Department of Medical Genetics (A.F.E., C.H.), Shodair Children's Hospital, Helena, MT; Division of Clinical Genomics (K.L.H.), Ambry

Genetics, Aliso Viejo, CA; Sektion für Neuropädiatrie und Stoffwechselmedizin (S.S.-B.), Zentrum für Kinder und Jugendmedizin, Heidelberg, Germany; Carol Davila University of Medicine Bucharest (D.C., A.S.), Department of Clinical Neuroscience, Pediatric Neurology Discipline; Pediatric Neurology Clinic (D.C., A.S.), Alexandru Obregia Hospital, Bucharest, Romania; Neurogenetics Group (T. Djémié, P.D.J., S.W., A.S.), Department of Molecular Genetics, VIB, Antwerp; Laboratory of Neurogenetics (T. Djémié, P.D.J., S.W.), Institute Born-Bunge, University of Antwerp, Belgium; Department of Medical Genetics (D.H.-Z.), Institute of Mother and Child, Warsaw, Poland; Department of Molecular Biology and Genetics (H.C.), Bogaziçi University, Istanbul, Turkey; Department of Neuropediatrics (I.H., H.M.), University Medical Center Schleswig-Holstein, Christian-Albrechts University, Kiel, Germany; Division of Neurology (I.H.), The Children's Hospital of Philadelphia, PA; Neurology Laboratory and Epilepsy Unit (J.S.), Department of Neurology, Instituto de Investigatión Sanitaria-Fundación Jiménez Díaz, Universidad Autónoma de Madrid; IIS-Fundación Jiménez Díaz and Centro de Investigación Biomédica en Red de Enfermedades Raras (J.S.), Madrid, Spain; Pediatric Neurology and Muscular Diseases Unit (P.S.), Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, and Maternal and Child Health, University of Genoa G. Gaslini Institute, Genova, Italy; Department of Neurology (P.D.J., S.W.), Antwerp University Hospital; GENOMED (A.S.), Center for Medical Genetics, University of Antwerp, Belgium; Department of Genetics (K.M.), United Laboratories, Tartu University Hospital; Department of Pediatrics (I.T., T.T.), University of Tartu; Child Neurology Unit of Children's Clinic of Tartu University Hospital (I.T., T.T.), Tartu, Estonia; Department of Pediatric Neurology and Developmental Medicine (I.B.), University of Munich; Center for Human Genetics and Laboratory Diagnostics (I.R.), Martinsried; and Institute of Human Genetics (J.R.L.), University of Leipzig Hospitals and Clinics, Leipzig, Germany. S.M. is currently affiliated with the Florey Institute of Neuroscience and Mental Health, Melbourne, Australia.

AUTHOR CONTRIBUTIONS

K. Johannesen: study concept and design, acquisition of data, analysis and interpretation. C. Marini: study concept and design, acquisition of data, analysis and interpretation. S. Pfeffer: study concept and design, acquisition of data, analysis and interpretation. R.S. Møller: study concept and design, acquisition of data, analysis and interpretation, study supervision. T. Dorn: acquisition of data. C. Niturad: acquisition of data. E. Gardella: analysis and interpretation. Y. Weber: acquisition of data, critical revision. M. Søndergård: acquisition of data. H. Hjalgrim: analysis and interpretation, study supervision. M. Nikanorova: acquisition of data. F. Becker: acquisition of data. L.H.G. Larsen: acquisition of data. H.A. Dahl: acquisition of data. O. Maier: acquisition of data. D. Mei: acquisition of data. S. Biskup: acquisition of data. K.M. Klein: acquisition of data, critical revision. P.S. Reif: acquisition of data. F. Rosenow: acquisition of data. A.F. Elias: acquisition of data. C. Hudson: acquisition of data. K.L. Helbig: acquisition of data, critical revision. S. Schubert-Bast: acquisition of data, critical revision. M.R. Scordo: acquisition of data. D. Craiu: acquisition of data. T. Djémié: acquisition of data. D. Hoffman-Zacharska: acquisition of data. H. Caglayan: acquisition of data. I. Helbig: acquisition of data. J. Serratosa: acquisition of data. P. Striano: acquisition of data. P. De Jonghe: acquisition of data. S. Weckhuysen: acquisition of data. A. Suls: acquisition of data. K. Muru: acquisition of data. I. Talvik: acquisition of data. T. Talvik: acquisition of data. H. Muhle: acquisition of data. I. Borggraefe: acquisition of data. I. Rost: acquisition of data. R. Guerrini: acquisition of data, critical revision. H. Lerche: acquisition of data, critical revision. J.R. Lemke: acquisition of data, analysis and interpretation, critical revision. G. Rubboli: acquisition of data, analysis and interpretation, critical revision, study supervision. S. Maljevic: acquisition of data, analysis and interpretation, critical revision.

ACKNOWLEDGMENT

The authors thank the patients and their families for participating in this study and Nicole Zapezauer for excellent technical assistance.

STUDY FUNDING

Part of this work was financed by the European Science Foundation Eurocores project EuroEPINOMICS (CoGIE) (DFG Le1030/11-1 to H.L., S.M., F.R.), by the Federal Ministry for Education and Research (BMBF, program on rare diseases, IonNeurONet: 01 GM1105A, to S.M., H.L.), the EU Seventh Framework Programme (FP7) under the project DESIRE grant agreement N602531 (to R.G.), and the Italian Ministry of Health, Grant Agreement N (to C.M.). The P.E. Kempkes Foundation (5/12 to P.S.R.). T. Djemie is a PhD fellow of the Institute of Science and Technology (IWT). T. Djemie, A.S., P.D.J., and S.W. were funded through the International Coordination Action (ICA) grant G0E8614N.

DISCLOSURE

K. Johannesen, C. Marini, S. Pfeffer, R. Møller, T. Dorn, C. Niturad, E. Gardella, Y. Weber, M. Søndergård, H. Hjalgrim, M. Nikanorova, F. Becker, L. Larsen, H. Dahl, O. Maier, D. Mei, S. Biskup, K. Klein, P. Reif, F. Rosenow, A. Elias, and C. Hudson report no disclosures relevant to the manuscript. K. Helbig is employed by and receives a salary from Ambry Genetics. S. Schubert-Bast, M. Scordo, D. Craiu, T. Djémié, D. Hoffman-Zacharska, H. Caglayan, I. Helbig, J. Serratosa, P. Striano, P. De Jonghe, S. Weckhuysen, A. Suls, K. Muru, I. Talvik, T. Talvik, H. Muhle, I. Borggraefe, I. Rost, R. Guerrini, H. Lerche, J. Lemke, G. Rubboli, and S. Maljevic report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received November 2, 2015. Accepted in final form June 3, 2016.

REFERENCES

1. Baulac S, Huberfeld G, Gourfinkel-An I, et al. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 2001;28:46–48.
2. Wallace RH, Marini C, Petrou S, et al. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 2001;28:49–52.
3. Kananura C, Haug K, Sander T, et al. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 2002;59:1137–1141.
4. Maljevic S, Krampfl K, Cobilanschi J, et al. A mutation in the GABAA receptor α1-subunit is associated with absence epilepsy. Ann Neurol 2006;59:983–987.
5. Feng HJ, Kang HQ, Song L, Dibbens L, Mulley J, Macdonald RL. Delta subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and surface expression of alpha4beta2delta GABAA receptors. J Neurosci 2006;26:1499–1506.
6. Epi4K Consortium, Epilepsy Phenome/Genome Project; Allen A, Berkovic SF, Cossette P, et al. De novo mutations in epileptic encephalopathies. Nature 2013;501:217–221.
7. Carvill G, Weckhuysen S, McMahon J, et al. *GABRA1* and *STXBP1*: novel genetic causes of Dravet syndrome. Neurology 2014;82:1245–1253.
8. Srivastava S, Cohen J, Pevsner J, et al. A novel variant in *GABRB2* associated with intellectual disability and epilepsy. Am J Med Genet A 2014;164A:2914–2922.
9. Cossette P, Liu L, Brisebois K, et al. Mutation of *GABRA1* in an autosomal dominant form of juvenile myoclonic epilepsy. Nat Genet 2002;31:184–189.
10. Yalçin Ö. Genes and molecular mechanisms involved in the epileptogenesis of idiopathic absence epilepsies. Seizure 2011;21:79–86.
11. Berg A, Berkovic S, Brodie M, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010;51:676–685.
12. Roth FC, Draguhn A. GABA metabolism and transport: effects on synaptic efficacy. Neural Plast 2012;2012:805830.
13. Lachance-Touchette P, Brown P, Meloche C, et al. Novel α1 and γ2 GABAA receptor subunit mutations in families

with idiopathic generalized epilepsy. Eur J Neurosci 2011; 34:237–249.
14. Klassen T, Davis C, Goldman A, et al. Exome sequencing of ion channel genes reveals complex variant profiles confounding personal risk assessment in epilepsy. Cell 2011; 145:1036–1048.
15. Rubinstein M, Han S, Tai C, et al. Dissecting the phenotypes of Dravet syndrome by gene deletion. Brain 2015;138(pt 8):2219–2233. doi: 10.1093/brain/awv142.
16. Macdonald RL, Kang JQ, Gallagher MJ. GABA(A) receptor subunit mutations and genetic epilepsies. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies [Internet], 4th ed. Bethesda, MD: National Center for Biotechnology Information (US); 2012. Available at: http://www.ncbi.nlm.nih.gov/books/NBK98205/. Accessed November 5, 2015.
17. Kash TL, Trudell JR, Harrison NL. Structural elements involved in activation of the gamma-aminobutyric acid type A (GABAA) receptor. Biochem Soc Trans 2004;32 (pt 3):540–546.
18. Arain FM, Boyd KL, Gallagher MJ. Decreased viability and absence-like epilepsy in mice lacking or deficient in the GABAA receptor alpha 1 subunit. Epilepsia 2012;53:e161–e165.
19. Raud S, Sütt S, Luuk H, et al. Relation between increased anxiety and reduced expression of alpha1 and alpha2 subunits of GABA(A) receptors in Wfs1-deficient mice. Neurosci Lett 2009;460:138–142.
20. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med 2015;18:696–704. doi: 10.1038/gim.2015.148.