

A novel *GABRG2* mutation associated with febrile seizures

Abstract—Mutations in the gene encoding the $\gamma 2$ subunit of the γ -aminobutyric acid type A receptor (*GABRG2*) have been reported to cause childhood absence epilepsy (CAE), febrile seizures (FS), and generalized epilepsy with FS plus (GEFS+). The authors analyzed *GABRG2* in 47 unrelated patients with CAE, FS, and GEFS+ and identified a novel mutation that cosegregated with FS. Electrophysiologic studies demonstrated altered current desensitization and reduced benzodiazepine enhancement in mutant receptors.

NEUROLOGY 2006;67:687–690

D. Audenaert, PhD*; E. Schwartz, BSc*; K.G. Claes, MD, PhD; L. Claes, PhD; L. Deprez, MSc; A. Suls, MSc; T. Van Dyck, BSc; L. Lagae, MD; C. Van Broeckhoven, PhD; R.L. Macdonald, MD, PhD; and P. De Jonghe, MD, PhD

Febrile seizures (FS; MIM 121210) account for the majority of childhood seizure disorders, affecting 2% to 5% of children before age 5 years. FS are usually benign, but 2% to 7% of children with FS develop epilepsy later in life. In 1997, Scheffer and Berkovic described the syndrome of generalized epilepsy with FS plus (GEFS+; MIM 604233).¹ GEFS+ patients have classic FS, FS that persist beyond age 5 years (FS plus, FS+), and subsequent afebrile seizures, including absences.

Mutations in the gene encoding the $\gamma 2$ subunit of the γ -aminobutyric acid type A (GABA_A) receptor (*GABRG2*) have been described in GEFS+.^{2,3} The GABA_A receptor is a ligand-gated chloride channel that consists of two α subunits, two β subunits, and one γ or δ subunit. The receptor complex contains two GABA -binding sites and a benzodiazepine-binding site. Two regions necessary for binding of benzodiazepines are present in the extracellular amino terminus of the $\gamma 2$ subunit. Electrophysiologic studies demonstrated that GEFS+-associated *GABRG2* mutations cause accelerated current deactivation or decreased current amplitude due to reduced membrane expression of GABA_A receptors.^{4,5} Identification of additional *GABRG2* disease alleles

and subsequent functional characterization will aid in our understanding of the contribution of *GABRG2* mutations to the pathophysiology of epilepsy.

Methods. We selected 47 unrelated patients with a diagnosis of FS (n = 14), GEFS+ (n = 22), or CAE (n = 11) and with multiple family members with seizures. Patients and their relatives were diagnosed according to the classification criteria of the International League Against Epilepsy.⁶ DNA samples were sent to our laboratory for further diagnostic genetic analysis for the known GEFS+ genes. As a control group, 184 unrelated individuals were randomly selected from the Belgian population. The Commission for Medical Ethics of the University of Antwerp approved this study, and participants or their legal representative signed an informed consent form.

We extracted genomic DNA from peripheral blood of patients using standard methods, and PCR amplified all exons and splice site junctions of *GABRG2*. We sequenced PCR products using the BigDye Terminator Cycle Sequencing kit from Perkin Elmer Applied Biosystems (Foster City, CA). Sequences were analyzed on an ABI 3730 automated sequencer. We used direct sequencing for segregation analysis and pyrosequencing to analyze genomic DNA of the control individuals.

Human GABA_A receptor subunits $\alpha 1$, $\beta 2S$, and $\gamma 2L$ were individually subcloned into expression vector pcDNA3.1. The $\gamma 2L$ subunit R139G mutation was made using the QuikChange kit and confirmed by DNA sequencing. HEK 293T fibroblasts were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum and maintained at 37°C. Using Fugene6, cells were cotransfected with human $\alpha 1$, $\beta 2$, $\gamma 2L$ (wild-type) or $\alpha 1$, $\beta 2$, $\gamma 2L$ (R139G) (homozygous) subunit complementary DNAs (cDNAs) at a ratio of 2:2:2 μg and with 1 μg of pHook-1 cDNA, which was used for immunomagnetic selection.⁷ Electrophysiologic recordings were made 18 to 24 hours after selection.

Cell attached or lifted whole cell recordings were obtained as described⁵ at -20 mV. The chloride equilibrium potential was approximately zero. Cells were rapidly superfused with GABA and drugs with an exchange time less than 1 millisecond. The current desensitization time course was fitted using the Levenberg-Marquardt least squares method. Only currents whose best fit had four time constants were kept for analysis. Data were represented as mean \pm SEM, and significance was determined using a Student's unpaired *t* test.

Results. We analyzed *GABRG2* in 47 unrelated patients with FS, GEFS+, or CAE. In one patient, we identified a heterozygous c.529C>G transversion in exon 4 of *GABRG2* that cosegregated with FS according to autosomal dominant inheritance with reduced penetrance (figure 1). One mutation carrier (I.1) was symptom free; no phenocopies were present. The mutated allele was not present in 368 control chromosomes. At the protein level, the c.529C>G transversion resulted in the substitution of a highly conserved arginine with glycine at position 139 in the mature

*These authors contributed equally to this work.

From the Neurogenetics Group, Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology, University of Antwerp, Antwerp, Belgium (D.A., K.G.C., L.C., L.D., A.S., T.V.D., C.V.B., P.D.J.); Neuroscience Graduate Program (E.S.) and Department of Neurology (R.L.M.), Vanderbilt University Medical Center, Nashville, TN; Division of Neurology, University Hospital of Antwerp, Antwerp, Belgium (K.G.C., P.D.J.); and Division of Child Neurology, University Hospital Gasthuisberg, Leuven, Belgium (L.L.).

Financial support was received from the Fund for Scientific Research Flanders (FWO-F), the Medical Foundation Queen Elisabeth, and the Interuniversity Attraction Poles program P19/5 of the Federal Science Policy Office, Belgium, and NIH training grant T32 MH64913 (E.S.). D.A. is a PhD fellow of the FWO-F.

Disclosure: The authors report no conflicts of interest.

Received December 23, 2005. Accepted in final form April 24, 2006.

Address correspondence and reprint requests to Dr. Peter De Jonghe, Neurogenetics Group, Department of Molecular Genetics University of Antwerp, Campus CDE Parking P4, Building V Universiteitsplein 1, 2610 Antwerp, Belgium; e-mail: peter.dejonghe@ua.ac.be

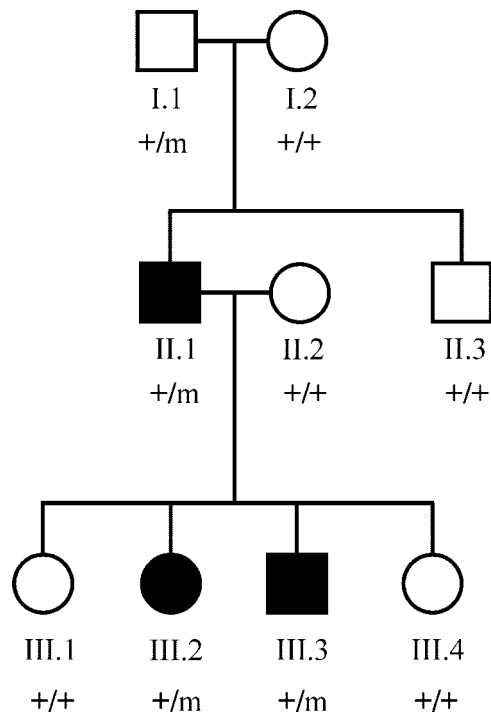


Figure 1. Pedigree of a family with a mutation in *GABRG2* (c.529C>G). Square = male; circle = female; open symbol = unaffected individual; filled symbol = patient with febrile seizures; + = wild-type allele; m = mutated allele.

peptide, localized in the second benzodiazepine-binding site of the $\gamma 2$ subunit.

The proband (III.2) of this three-generational family (figure 1) was a 7-year-old girl who had her first generalized tonic-clonic FS at age 13 months and her last one at age 3.5 years. In total, she experienced 10 seizures, which were all provoked by fever. Antiepileptic drug treatment (valproic acid) was started after her second episode of FS. Her 5-year-old brother (III.3) experienced a similar onset, frequency, and presentation of generalized tonic-clonic FS. He was also treated with valproic acid. In both sib-

lings, the FS had a longer duration and presented more severely before age 2 years. At 1.5 years, the brother presented once with status epilepticus during 60 minutes, which was treated with IV diazepam (DZP). Their father (II.1) had a first tonic-clonic seizure associated with fever at the age of 18 months. Later he experienced 20 to 25 additional FS, the last one at age 4 years. He was treated with antiepileptic drugs. Neither grandparents (I.1 and I.2) had a history of seizures. All patients had normal mental development and did not develop epilepsy later in life.

Currents were evoked by 2-second applications of 1 mM GABA to cell attached or “lifted” cells (figure 2A). The average peak current of mutant receptors was not different from that of wild-type receptors (data not shown); however, mutant receptor currents desensitized more rapidly than wild-type receptor currents. There was an increased contribution of the fast component of desensitization in mutant receptors, whereas there was no difference between the time constants (figure 2, B and C).

We determined the extent of benzodiazepine enhancement of wild-type and mutant receptors by applying 1 μ M GABA with or without 1 μ M DZP (figure 3A). Mutant currents had significantly reduced sensitivity to 1 μ M DZP relative to wild-type receptors (figure 3B).

To eliminate the possibility that these results were due to expression of fast desensitizing, DZP-insensitive and highly zinc-sensitive $\alpha\beta$ receptors,⁸ zinc sensitivity of the mutant receptors was determined. There was no significant difference in zinc inhibition between the wild-type and mutant receptors (figure 3, C and D), confirming the presence of $\alpha\beta\gamma$ receptors containing the mutant $\gamma 2$ (R139G).

Discussion. We analyzed *GABRG2* in 47 unrelated patients with FS, GEFS+, or CAE and with a family history of seizures. We identified a novel mutation (p.R139G) and extended the spectrum of *GABRG2* mutations that result in FS. Interestingly, the mutation produced FS only and no other epilepsy seizure types. This differs from the considerable in-

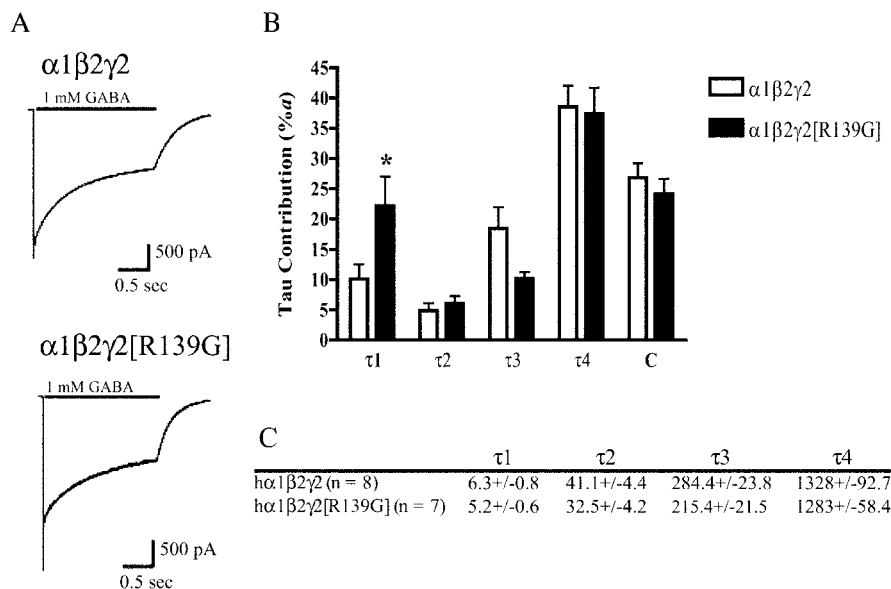


Figure 2. The R139G mutation results in altered desensitization of the receptor. (A) Representative traces of a 2-second γ -aminobutyric acid (GABA) application to lifted HEK293T cells expressing wild-type (top) or mutant receptors (bottom). (B) Relative to wild-type receptors, mutant receptor currents had an increased contribution of the fast component, $\tau 1$, of current desensitization. * Indicates difference ($p < 0.05$). The difference in contribution of $\tau 3$ had a p value of 0.545. (C) Table of the average fitted time constants (τ in milliseconds). There were no significant differences in any of the time constants of desensitization between the wild-type and mutated receptor.

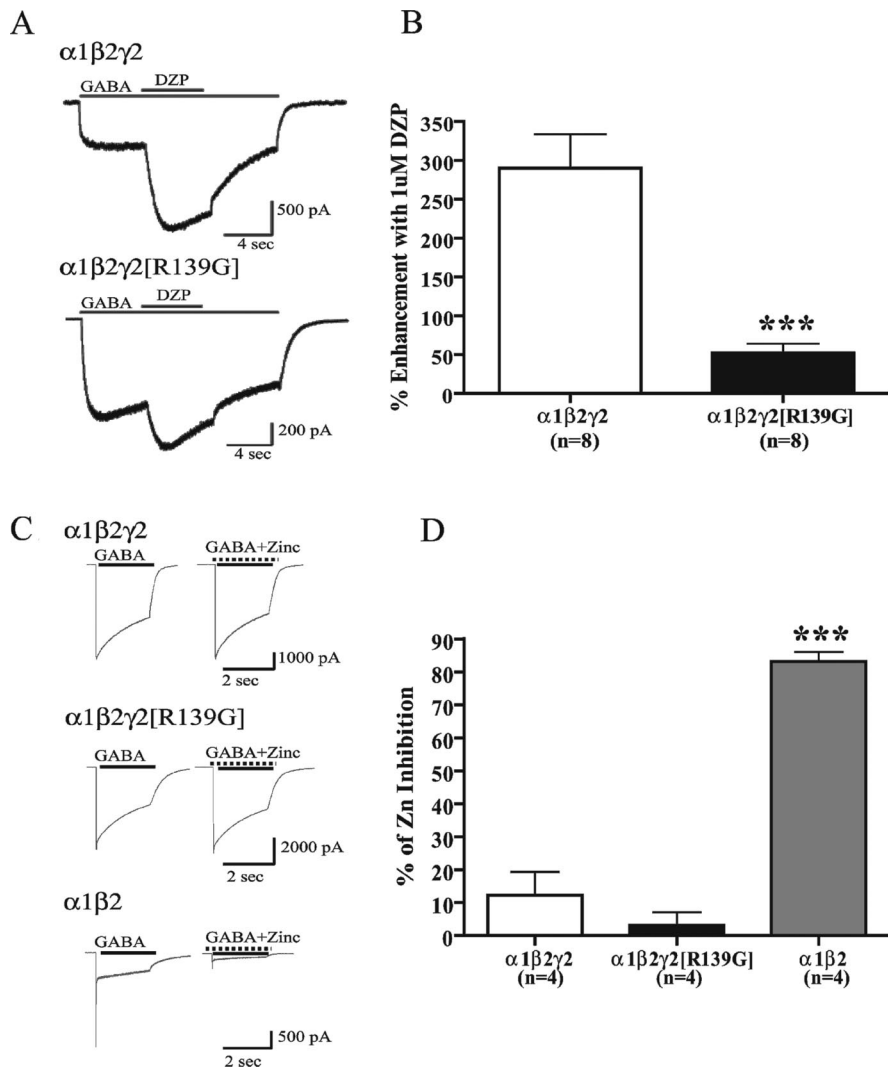


Figure 3. The R139G mutation results in reduced diazepam (DZP) sensitivity but is incorporated into receptor pentamer. (A) Representative traces of DZP enhancement of mutant and wild-type receptors. (B) Mutant receptors had significantly reduced benzodiazepine sensitivity compared with wild-type receptors when agonist and modulator are applied to lifted cells. *** Indicates difference ($p = 0.0001$). Enhancement was calculated as $(I_{GABA} - I_{GABA + DZP}) / I_{GABA} * 100$. (C) Representative traces of current evoked from attached cells in which $10 \mu M Zn^{2+}$ was preapplied before coapplication with a $1-mM \gamma$ -aminobutyric acid (GABA) pulse show that the R139G mutation was insensitive to Zn^{2+} inhibition, indicating that the mutant subunit was expressed on the cell surface as an $\alpha 1\beta 2\gamma 2(R139G)$ pentamer ($n = 4$ for each condition). (D) The Zn^{2+} sensitivity of the R139G mutant was not different from the wild-type receptor, indicating that the benzodiazepine insensitivity was not due to reduced expression of the mutant $\gamma 2$ subunit. *** Indicates difference from wild type ($p < 0.0001$).

trafamilial and interfamilial phenotypic variability associated with previously reported *GABRG2* mutations, ranging from FS and CAE to severe myoclonic epilepsy of infancy.^{2,3,9} However, because of the limited pedigree size in our study, it was not possible to make conclusive genotype–phenotype correlations. Segregation analysis showed that individual I.1 carried the mutation and did not present with FS, illustrating that the c.529C>G mutation was not a fully penetrant allele. However, no parental information was available to recollect possible seizure history for individual I.1, and it cannot be excluded that he had an undocumented FS. No disease-causing *GABRG2* mutations were identified in the other 46 patients, illustrating that *GABRG2* does not play a major role in FS, GEFS+, and CAE.

We demonstrated that the $\gamma 2(R139G)$ mutation incorporated into $GABA_A$ receptors and conferred altered current desensitization. The fast phase of desensitization is the primary contributor to the shape of an inhibitory postsynaptic current (IPSC).¹⁰ Increased fast phase desensitization should result in decreased IPSC amplitude and, therefore, disinhibition leading to seizures.

The p.R139G missense mutation is localized in one of two benzodiazepine-binding sites in the $\gamma 2$ subunit. Whether the reduced sensitivity of the mutation to benzodiazepines is due to altered binding or transduction remains to be determined.

Acknowledgment

The authors thank the patients and their relatives for their participation. The authors also thank the VIB Genetic Service Facility for the genetic analyses, Iris Smouts for collecting blood samples, and Ningning Hu for technical assistance in creating cDNAs.

References

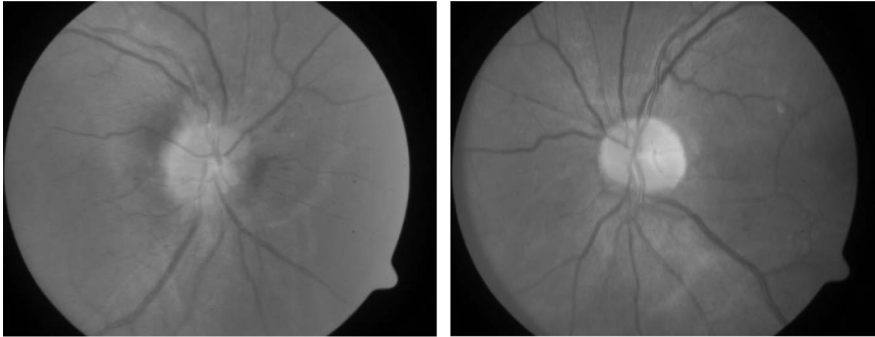
- Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus: a genetic disorder with heterogeneous clinical phenotypes. *Brain* 1997;120:479–490.
- Baulac S, Huberfeld G, Gourfinkel-An I, et al. First genetic evidence of $GABA(A)$ receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat Genet* 2001;28:46–48.
- 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.
- Bianchi MT, Song L, Zhang H, Macdonald RL. Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. *J Neurosci* 2002;22:5321–5327.
- Kang JQ, Macdonald RL. The $GABA_A$ receptor gamma2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures

causes retention of alpha1beta2gamma2S receptors in the endoplasmic reticulum. *J Neurosci* 2004;24:8672–8677.

- Engel J Jr. International League Against Epilepsy (ILAE): a proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 2001;42:796–803.
- Greenfield LJ Jr, Sun F, Neelands TR, Burgard EC, Donnelly JL, MacDonald RL. Expression of functional GABA_A receptors in transfected L929 cells isolated by immunomagnetic bead separation. *Neuropharmacology* 1997;36:63–73.

- Boileau AJ, Baur R, Sharkey LM, Sigel E, Czajkowski C. The relative amount of cRNA coding for gamma2 subunits affects stimulation by benzodiazepines in GABA(A) receptors expressed in *Xenopus* oocytes. *Neuropharmacology* 2002;43:695–700.
- Harkin LA, Bowser DN, Dibbens LM, et al. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet* 2002;70:530–536.
- Jones MV, Westbrook GL. The impact of receptor desensitization on fast synaptic transmission. *Trends Neurosci* 1996;19:96–101.

NeuroImages



right

left

Figure 1. Retinal photograph showing severe hypertensive changes confined to the right fundus.

Unilateral malignant hypertensive retinopathy

T. Umapathi, MBBS (Singapore), FRCP (UK); and Swee-Ling Wong, MBBS (UK), BA, Singapore

A 42-year-old man, whose blood pressure averaged 230/130 mm Hg, presented with right brachiofacial weakness from acute left internal watershed infarctions. Funduscopy revealed severe hypertensive changes, disc edema, arteriovenous nicking, and silver wiring, confined to right eye (figure 1). Visual acuity was normal. There was no Horner's syndrome or neck pain. Neck MR scans showed severe narrowing of left internal carotid artery, from origin to the skull base, suggestive of carotid artery dissection (figure 2). The resulting reduced blood flow protected the left retina from effects of severe hypertension.¹ Accelerated hypertension was the likely cause of carotid artery dissection.²

Copyright © 2006 by AAN Enterprises, Inc.



Figure 2. MR scan of the neck showing severe narrowing of the left internal carotid artery, "string sign," extending from its origin to the skull base.

Disclosure: The authors report no conflicts of interest.

Address correspondence and reprint requests to Dr. Umapathi, National Neuroscience Institute, 11 Jalan Tan Tock Seng, Singapore 308433; e-mail: umapathi@nni.com.sg

- Wong TY, Mitchell P. Hypertensive retinopathy. *N Engl J Med* 2004;351:2310–2317.
- Caplan LR, Bioussé V. Cervicocranial arterial dissections. *J Neuroophthalmol* 2004;24:299–305.

Neurology[®]

Unilateral malignant hypertensive retinopathy

T. Umapathi and Swee-Ling Wong

Neurology 2006;67;690

DOI 10.1212/01.wnl.0000218187.48702.3a

This information is current as of August 21, 2006

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/67/4/690.full
References	This article cites 2 articles, 0 of which you can access for free at: http://n.neurology.org/content/67/4/690.full#ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Epilepsy/Seizures http://n.neurology.org/cgi/collection/all_epilepsy_seizures Ion channel gene defects http://n.neurology.org/cgi/collection/ion_channel_gene_defects
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright . All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

