

## FULL-LENGTH ORIGINAL RESEARCH

# Exon-disrupting deletions of *NRXN1* in idiopathic generalized epilepsy

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## SUMMARY

**Purpose:** Neurexins are neuronal adhesion molecules located in the presynaptic terminal, where they interact with postsynaptic neuroligins to form a transsynaptic complex required for efficient neurotransmission in the brain. Recently, deletions and point mutations of the neurexin I (*NRXN1*) gene have been associated with a broad spectrum of neuropsychiatric disorders. This study aimed to investigate if *NRXN1* deletions also increase the risk of idiopathic generalized epilepsies (IGEs).

**Methods:** We screened for deletions involving the *NRXN1* gene in 1,569 patients with IGE and 6,201 controls using high-density oligonucleotide microarrays.

**Key Findings:** We identified exon-disrupting deletions of *NRXN1* in 5 of 1,569 patients with IGE and 2 of 6,201 control individuals ( $p = 0.0049$ ; odds ratio (OR) 9.91, 95% confidence interval (CI) 1.92–51.12). A complex familial segregation pattern in the IGE families was observed, suggesting that heterozygous *NRXN1* deletions are susceptibility variants. Intriguingly, we identified a second large copy number variant in three of five index patients, supporting an involvement of heterogeneous susceptibility alleles in the etiology of IGE.

**Significance:** We conclude that exon-disrupting deletions of *NRXN1* represent a genetic risk factor in the genetically complex predisposition of common IGE syndromes.

**KEY WORDS:** Idiopathic generalized epilepsy, 1q21.1 microdeletion, Two-hit hypothesis, *NRXN1*.

Idiopathic generalized epilepsies (IGEs) including childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and

epilepsies with generalized tonic-clonic seizures (EGTCS) alone affect 0.2% of the general population and account for up to 30% of all epilepsies (International League Against Epilepsy, 1989; Jallon et al., 2001). Genetic factors play a predominant role in the etiology of these IGE syndromes with heritability estimates of >80%, and recurrence risk for first-degree relatives varying between 4% and 8% (Helbig et al., 2008). However, despite extensive research, the genetic variants predisposing to the common IGE syndromes largely remain elusive.

Accepted November 14, 2012; Early View publication January 7, 2013.

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The predisposing role of copy number variations (CNVs) in human diseases, especially in neurodevelopmental and psychiatric disorders, is becoming increasingly evident. CNVs such as microdeletions at 15q11.2, 15q13.3, and 16p13.11 predispose to IGE (Helbig et al., 2009; de Kovel et al., 2010) and are also strongly associated with schizophrenia (Stefansson et al., 2009; Ingason et al., 2011), intellectual disability (ID; Sharp et al., 2008; Hannes et al., 2009; Burnside et al., 2011), and autism spectrum disorder (ASD; Pagnamenta et al., 2009), but can also be present in healthy individuals. One explanation for this is that CNVs can disrupt the homeostasis of normal neuronal development and lead to a range of disorders as part of a neurodevelopmental continuum (Coe et al., 2012a,b). Accordingly, the question arises whether additional CNVs associated with neurodevelopmental disorders are also risk factors for IGE. Neurexins are neuronal adhesion molecules located in the presynaptic terminal where they interact with postsynaptic neuroligins to form a transsynaptic complex, which is required for synaptic contacts and efficient neurotransmission in the brain (reviewed by Sudhof, 2008). Recently, heterozygous exon-disrupting deletions and truncating point mutations of *NRXN1* have been associated with a broad spectrum of neurodevelopmental and psychiatric disorders including schizophrenia, ASD, and ID (Kirov et al., 2009; Rujescu et al., 2009; Ching et al., 2010). Comorbid epilepsy has been reported in a few of these cases (Ching et al., 2010; Gregor et al., 2011). In addition, compound *NRXN1* mutations consisting of a combination of heterozygous exon-disrupting microdeletions and nonsense or splice-site mutations have recently been described in patients with severe early onset epilepsy and profound ID (Harrison et al., 2011; Duong et al., 2012). The present association study aimed to investigate if exon-disrupting deletions of *NRXN1* increases risk of common IGE syndromes.

## METHODS

### Study participants

The present candidate gene CNV study included a case–control sample comprising 1,569 unrelated IGE patients (600 male/969 female) of European ancestry (patients collected from epilepsy centers in Austria, *n* = 197; Belgium, *n* = 53; Denmark, *n* = 95; Germany, *n* = 933; and The Netherlands, *n* = 291) (EPICURE Consortium et al., 2012) and 6,201 unselected German controls, which all were typed by the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, U.S.A.). Phenotyping and classification of IGE syndromes were carried out according to standardized phenotyping protocols. According to the exclusion criteria, IGE patients with a history of severe major psychiatric disorders (ASD, schizophrenia, affective disorder: recurrent episodes requiring pharmacotherapy or treatment in a hospital), or moderate to severe intellectual disability (no basic education, permanently requiring

professional support in their daily life) were excluded. The epilepsy patients comprised the following IGE syndromes: 693 CAE and JAE; 625 JME and 251 patients with EGTCS alone. The control cohort comprised 6,201 unselected German individuals provided by the PopGen biobank (University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany), the KORA (Cooperative Health Research in the Region of Augsburg) research platform, and the SHIP Consortium representing epidemiologically recruited cohorts from the Northern (Schleswig, PopGen, *n* = 1,163), Southern (Augsburg, KORA, *n* = 1,625) and Northeastern (Greifswald, SHIP, *n* = 3,413) regions of Germany. The population controls were not screened for epilepsy or major psychiatric disorders; consequently a small proportion (<1%) of the controls might be affected by these disorders. All study participants gave written informed consent according to the regulations at their local institutional review boards.

### Genotyping and CNV detection

The DNA samples were typed for 1.8 million probe sets on the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix SNP 6.0 array). CNV analysis of all Affymetrix SNP 6.0 arrays was performed by the algorithm implemented in the Affymetrix Genotyping Console version 4.0 at the Cologne Center for Genomics. Segments with >20 markers and >40 kb in size were considered as highly confident CNV calls, even when the Affymetrix SNP 6.0 arrays were processed at different laboratories (Pinto et al., 2011). Changes of the log<sub>2</sub> signal intensity ratios of the *NRXN1* deletions were visually inspected to exclude technical artifacts. All exon-disrupting *NRXN1* deletions identified in the patient cohort were verified by TaqMan quantitative polymerase chain reaction (qPCR) (*NRXN1* exon 4 Hs04683030; Applied Biosystems, Foster City, CA, U.S.A.) and/or array comparative genomic hybridization (CGH). For segregation analysis, all available family members were typed by real-time qPCR and/or by array CGH (SurePrint G3 Human CGH Microarray Kit, 4x180K; Agilent Technologies, Santa Clara, CA, U.S.A.). Based on the estimated genomic positions from the array we also looked for purely intronic deletions in cases versus controls.

*NRXN1*-deletion carriers were examined for other large CNVs using the following criteria: (1) CNV should be ≥ 0.5 Mb in size, and (2) at least one brain expressed RefSeq gene. The CNV should be overlapping a published recurrent CNV associated with epilepsy, neurodevelopmental, or neuropsychiatric disorder. Alternatively, the CNV should be ≥ 0.5 Mb in size, and contain a gene already associated with epilepsy, neurodevelopmental, or neuropsychiatric disease, or at least one brain expressed RefSeq gene. The CNVs were verified by TaqMan qPCR and/or array CGH. The 1q21.1 microdeletion was validated by the use of a custom oligonucleotide array (Agilent Technologies) with 6,453 probes in the 1q21.1 region (chr1:142,000,000–148,000,000 hg18/Build 36) with average probe spacing of 849 bp.

### Statistical analysis

Association analysis between genotype and phenotype was carried out by two-tailed Fisher's exact test.

## RESULTS

We identified heterozygous exon-disrupting deletions of *NRXN1* in 5 (0.3%) of 1,569 patients with IGE (Fig. 1), whereas 2 (0.03%) were observed in 6,201 control individuals ( $p = 0.0049$ ; odds ratio [OR] 9.91, 95% confidence interval [CI] 1.92–51.12). We also looked for purely intronic deletions in cases versus controls; however, no statistically significant difference was found (5 of 1,569 IGE patients and 14 of 6,201 controls;  $p = 0.56$ ; Fisher's exact test, two-tailed). A lower, yet significant association between IGE and all types of *NRXN1* deletions in cases versus controls were also observed (10 of 1,569 IGE patients vs. 16/6,201 controls;  $p = 0.027$ ; Fisher's exact test, two-tailed).

The association between IGE and exon-disrupting *NRXN1* deletions is further strengthened if added control data from the CNV study published by the international schizophrenia consortium (1/3,181 controls; International Schizophrenia Consortium, 2008) together with public available CNV data from two independent control cohorts performed by Itsara et al. (2009) (0 of 2,493 population controls) and by Shaikh et al. (2009) (2 of 2,026 population controls). If adding these data we find exon-disrupting deletions of *NRXN1* in 5 of 1,569 IGE patients versus 5 of 13,901 controls ( $p = 0.0017$ ; Fisher's exact test, two-tailed; see also Figs. S1 and S2).

Of the five IGE patients with *NRXN1* deletions, two were affected by CAE, one by JAE, one by JME, and one by EGTS alone. All five deletions included the promoter region and/or one or more of the first exons of *NRXN1* (Fig. 1).

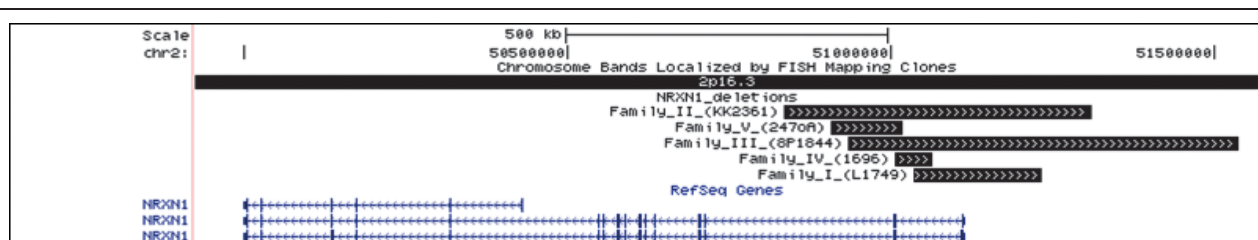
### Segregation analysis of the *NRXN1* deletions

The inheritance of the exon-disrupting *NRXN1* deletions was tracked in all five IGE families (Fig. 2). The deletion arose de novo in two patients (families IV and V), one was inherited from an affected parent (family I), and two index patients inherited the deletion from an unaffected parent

(families II and III). We observed four multiplex families with two or more affected individuals with IGE. In total, we observed 11 deletion carriers within the 5 families, 7 of those with IGE. Paternity and familial relationship was ensured by consistent Mendelian inheritance of single nucleotide polymorphism/short tandem repeat (SNP/STR) markers.

*Family I* was of German ancestry and consisted of three affected family members and the unaffected father (Fig. 2). The index patient (L1748) was a 29-year-old male. He presented with CAE at the age of 3 years. He had up to 12 absences per day and was treated with valproic acid (VPA). Electroencephalography (EEGs) showed typical 3 Hz spike and wave complexes. From the age of 7 on, he became seizure-free and is currently seizure-free without medication. In addition, he had two to three febrile seizures around 4 years of age. Neuropsychological testing performed at 6 years of age showed borderline intelligence (IQ = 74, Kaufman-Assessment Battery for Children K-ABC) with moderate learning disability. His sister had CAE from 6 to 10 years of age with up to five to eight evident clinical absences per day before treatment with VPA. Ictal EEGs were not available and interictal EEGs were normal. At the age of 1 year, she had a single febrile seizure. She showed a normal psychomotor development (a detailed neuropsychological testing was not performed) and is currently seizure-free without antiepileptic medication. The mother had unclear episodes of short unresponsiveness in her childhood, but additional diagnostic or therapeutic steps were not performed. We classified those episodes as possible absences in childhood. All three affected family members carried the 200-kb *NRXN1* deletion (chr2: 51.03–51.23 Mb, hg18).

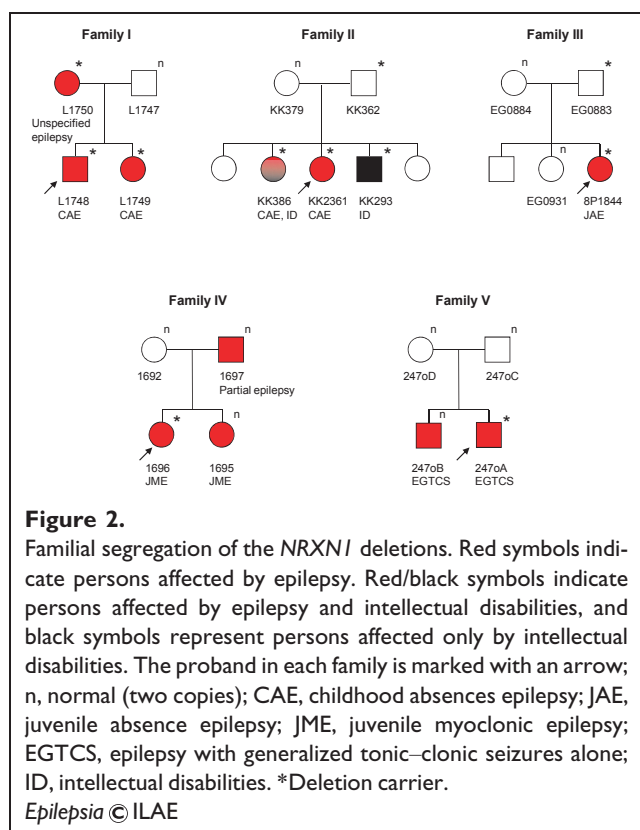
*Family II* was of German ancestry and consisted of five children, two of whom were affected by IGE (Fig. 2). Their mother was healthy and the father had type 1 diabetes. The index patient (KK2361) was a 10-year-old girl. She presented with CAE at the age of 5. The patient was initially treated with topiramate (TPM), leading to an aggravation of seizures; lamotrigine (LTG) therapy failed as well. The absences were controlled after introduction of VPA. EEG showed typical 3Hz spike and wave complexes up to 21 s. Neuropsychological testing performed at the age of



**Figure 1.**

Genomic positions of the five different *NRXN1* deletions. The deletions are indicated by the black bars. The figure was produced with the UCSC Genome Browser (<http://www.genome.ucsc.edu>) hg18 based on the genomic positions from the Affymetrix Genome-Wide Human SNP Array 6.0.

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7.5 years showed normal intelligence (practical intelligence IQ = 95 [normal range 85–115] and abstract intelligence score = 10 [normal range 7–13], HAWIVA III (Hannover-Wechsler-Intelligenztest für das Vorschulalter). Her older sister had absences from the age of 7–9 and was treated with sulthiame. At 9 years of age, EEG showed irregular spike and wave discharges and photosensitivity, but no seizures. Anticonvulsant therapy was withdrawn and the girl is currently seizure-free without medication. Neuropsychological testing at the age of 13 showed moderate ID (assessed with CMM, a subtest of the test battery for disabled children [TBGB] and with HAWIVA III with test results corresponding to a 7-year-old child. Arithmetic capabilities assessed with K-ABC showed IQ = 67, [normal range 85–115]). A younger brother had moderate ID (based on clinical observation) but no epilepsy. Parents had normal intelligence and normal EEG recordings. The 480 kb *NRXN1* deletion (chr2: 50.83–51.31 Mb, hg18) was detected in all three affected children and in the father unaffected with neurologic disease.

**Family III** was of Danish ancestry and consisted of a female with epilepsy and her healthy parents and sister (Fig. 2). The index patient (8P1844) was 30 years old and her epilepsy started with absences at age 11. From age 24 she also had a total of seven generalized tonic-clonic seizures on awakening (epilepsy with grand mal seizure on awakening, EGMA). At variance with typical juvenile absence epilepsy, her absences ended with a vocalization (“t-t-t”), which was noticed by both herself and those in her

surroundings. More recently, during some absences she reported a feeling of jerking of the arms which, however, was not visible in a video registration. Furthermore, the absences were resistant to treatment with VPA, LTG, levetiracetam (LEV), TPM, and ethosuximide. The ictal EEG, however, showed 3 Hz spike and waves, consistent with a diagnosis of JAE. Currently, she is treated with VPA and LTG and has no GTCS, but her absences continue with reduced frequency. She is diagnosed as IGE but not a typical case of JAE. She had a normal intelligence but was reported to have a fragile personality. The 600 kb *NRXN1* (chr2: 50.93–51.53 Mb, hg18) deletion was inherited from the unaffected father and not present in the unaffected sister. A paternal half-sister of the proband had schizophrenia and Attention Deficit/Hyperactivity Disorder (ADHD); however, she was not available for testing.

**Family IV** was a multiplex IGE family of German ancestry including two affected sisters and their affected father (Fig. 2). All three affected individuals had normal intelligence. The index patient (1,696) was a 45-year-old woman with JME characterized by typical bilateral myoclonic seizures of the arms and shoulders on awakening, with onset at 14 years of age. In addition, she experienced GTCS on awakening. The EEG showed generalized polyspike and wave discharges. Likewise, her 42-year-old sister had JME displaying characteristic bilateral myoclonic seizures and a single GTCS on awakening, with age of onset at 14 years. The interictal EEG showed generalized polyspike and wave discharges. In both patients, the psychomotor development appeared clinically normal; a neuropsychological testing was not performed. The 74-year-old father had partial epilepsy starting at the age of 57 years. His seizures initiated with a visual aura followed by secondarily GTCS. His interictal EEG was normal. The 70-year-old mother had no history of epileptic seizures and her EEG was normal. One clinically unaffected brother and one unaffected sister were not examined. In this family a 60-kb deletion (chr2: 51.00–51.06 Mb, hg18) occurred de novo in the proband.

**Family V** consisted of two affected siblings and their unaffected parents of Turkish origin (Fig. 2). Both affected siblings had normal intellectual development. The index patient (247oA) was a 31-year-old man. He presented with a photosensitive EGTCs at the age of 16 years with about seven seizures per month. EEG showed generalized spike and wave paroxysms. Under the treatment with TPM and LEV a significant seizure reduction (to approximately 1 seizure per month) was achieved. His 3 years older, more mildly affected brother also had EGTCs. He is currently under phenytoin treatment and has been seizure-free for 7 years. In this family the 110 kb *NRXN1* deletion (chr2: 50.90–51.01, hg18) arose de novo in the index patient.

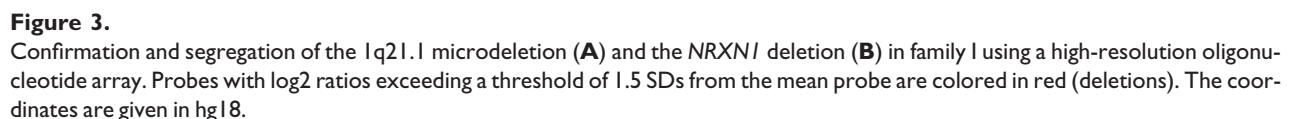
In summary, a *NRXN1* deletion was found in families I, II, and III in all affected family members (including one member with ID only) and two unaffected family members



including 18 RefSeq genes. This duplication arose de novo in the index patient, as it was not present in either parent or in the affected siblings. A 500-kb duplication at 10q23.31 (92.07–92.59 hg18) partially including exon 2–4 of the *HTR7* gene was detected in the proband of family V (inheritance not tested).

## DISCUSSION

In the present study, we investigated whether deletions of *NRXN1* increase the risk of common IGE syndromes. We detected deletions of the promoter region and/or the first exons of *NRXN1* in 5 of 1,569 individuals with IGE, corresponding to a frequency of 0.3% and in 2 of 6,201 controls (0.03%). The association with the IGE phenotype was significant ( $p = 0.0049$ ; OR 9.91, 95% CI 1.92–51.12). Taking into account that our population-based association



analysis compared the frequency of *NRXN1* deletions in IGE patients of European origin with that observed in German population controls (EPICURE Consortium et al., 2012), it might be possible that confounding by population stratification might affect the present association result. However, this potential bias is unlikely to play a substantial role, considering that similar frequencies of exon-disrupting *NRXN1* microdeletions have been reported in three cohorts of mainly Caucasian/European population controls (total: 3 of 7,700 = 0.039%; 1/3,181; International Schizophrenia Consortium, 2008; 0 of 2,493; Itsara et al., 2009; 2 of 2,026 (Shaikh et al., 2009). We also looked for purely intronic *NRXN1* deletions in cases versus controls; however, no statistically significant difference was found, which corresponds to the findings in studies of patients with schizophrenia (Rujescu et al., 2009).

There has been a remarkable phenotypic variability for individuals with an *NRXN1* deletion, ranging from apparently unaffected carriers to individuals with severe cognitive deficits, ASD, and schizophrenia. The present epilepsy cohort was ascertained by the IGE phenotype, excluding those patients affected by severe ID and major psychiatric disorder such as ASD and schizophrenia. None of the index patients had any psychiatric disorder, and four of the five index patients had normal intelligence, whereas one had borderline intelligence. It is therefore unlikely that the excess of exonic *NRXN1* deletions found in the present study is caused by unobserved comorbidity of IGE and psychiatric disorders or severe intellectual disability.

The human neurexin gene family consists of three genes—*NRXN1*, *NRXN2*, and *NRXN3*—that are subject to alternative promoter usage and extensive alternative splicing (Rowen et al., 2002). From each gene, two major isoforms,  $\alpha$  and  $\beta$ , are produced. The longer  $\alpha$ -form is transcribed from a promoter upstream of exon 1, and the shorter  $\beta$ -form is transcribed from a promoter located intragenic.  $\alpha$ -neurexins are essential for functional organization of synapses, and knockout mice lacking the  $\alpha$ -neurexins die shortly after birth (Missler et al., 2003), indicating a vital function of  $\alpha$ -neurexins in mammals. All five exonic deletions identified in the IGE patients include the promoter region and/or one or more of the first exons of *NRXN1*, leading to a disruption of the  $\alpha$  isoform of the gene.

The inheritance of the exon-disrupting *NRXN1* deletions was tracked in all five IGE families. The deletion arose de novo in two patients, two were inherited from unaffected parents, and one was inherited from an affected parent. However, the *NRXN1* deletion did not account for all the epilepsy risk in these families, as there were several individuals affected with epilepsy without the deletion and two unaffected individuals with the deletion, suggesting that the *NRXN1* deletion, like other microdeletions associated with IGE, is a susceptibility variant, rather than segregating in a monogenic fashion. Therefore, *NRXN1* deletions alone are neither necessary nor sufficient to cause IGE. In our three

families, the exon-disrupting *NRXN1* deletion showed an incomplete penetrance of 78%. Compared to the previous reports of *NRXN1* deletions in ID families, the penetrance in our families seems to be slightly higher: 8 of 12 mutations carriers were reported to be affected (Ching et al., 2010; 67% penetrance) or 5 of 10 were healthy (Gregor et al., 2011, 50% penetrance).

Of interest, we identified a second large CNV (>500 kb) in three of five index patients (60%) and in none of the unaffected *NRXN1* carriers not the two controls or in the unaffected family members. This frequency of large CNVs in affected deletion carriers is higher than would be expected in the general population, although the comparability between datasets is limited to the different genotyping platforms used. Girirajan et al. (2010) found a CNV >500 kb in 8.7% (271 of 2,493) of healthy controls. Using this frequency, the presence of an additional CNV >500 kb in three of five individuals carrying an exon-disrupting *NRXN1* deletion would be expected only once in 174 random binominal distributions ( $p = 0.0057$ ). An increased frequency of second CNVs has been found in some rare variants associated with ID, which led to the suggestion of a “two-hit hypothesis” (Girirajan et al., 2010). This hypothesis entails that much of the individual disease risk for a given neurodevelopmental disorder is conferred by a pathogenic CNV in conjunction with a second, independent pathogenic variant (Girirajan et al., 2010). Herein, we suggest that for IGE this two-hit model might fit to the polygenic heterogeneity model for common epilepsy syndromes as proposed by Mulley et al. (2005). The model states that “common epilepsies with complex genetics are caused by heterogeneous but pathogenic subsets of susceptibility alleles drawn from a much larger pool of potential susceptibility genes, meaning variation at no individual susceptibility gene is necessary or sufficient for seizures” (Mulley et al., 2005; Dibbens et al., 2007).

Although it was previously suggested that a second hit might also lead to more severe phenotype (Girirajan et al., 2010), the existence of a second independent possibly pathogenic CNV in patients with IGE observed in this study, suggests that this mechanism might also apply to relatively mild phenotypes.

In family I, in the present study, we found that a very rare recurrent microdeletion at 1q21.1 cosegregated with the *NRXN1* deletion and with epilepsy in this family. The 1q21.1 microdeletion itself has been associated with a broad range of neurodevelopmental and psychiatric disorders including mild ID, ASD, ADHD, and IGE (Brunetti-Pierri et al., 2008; Mefford et al., 2008; de Kovel et al., 2010). In addition, some studies have reported dysmorphic features in 1q21.1 deletion carriers (Brunetti-Pierri et al., 2008; Mefford et al., 2008), which were not observed in the family presented here. Furthermore, patients with a 1q21.1 microdeletion have been shown to have a 40-fold enrichment for “double hits” when compared to controls (Girirajan et al., 2010).

The role of the other two “second hits” in *NRXN1* deletion carriers is less evident. In family II, we identified a de novo microduplication at 2q13–q14.1 in the index patient. The duplication involves 18 RefSeq genes not previously associated with epilepsy or ID.

Family V had a 500-kb duplication at 10q23.31 including exon 2–4 of the *HTR7* gene in addition to the *NRXN1* deletion in the index patient. *HTR7* encodes a G protein–coupled receptor for serotonin (5-HT<sub>7</sub>). The role of 5-HT<sub>7</sub> receptor in psychiatric and neurologic disorders has been investigated, but remains unclear (reviewed by Hedlund, 2009).

In contrast to rare monogenic IGE cases, most cases of IGE are considered polygenic. So far, the best model for the genetic basis of IGE is the polygenic heterogeneity model for common epilepsies. Our data support this model for the development of IGE.

In conclusion, we observed a significant excess of IGE patients carrying specifically an exon-disrupting deletion of *NRXN1* compared to controls. The deletion also acts in concert with other factors to modify neurologic phenotypes. There has been a remarkable phenotypic variability for individuals with a *NRXN1* deletion, ranging from apparently unaffected carriers to individuals with cognitive deficits, ASD, and schizophrenia. Systematic screening for additional pathologic variants through array CGH studies or massive parallel sequencing studies may reveal additional modifying variants that explain the high variability of phenotypes.

## ACKNOWLEDGMENTS

We thank all participants and their families for participating in this study. This work was supported by grants from the European Community (FP6 Integrated Project EPICURE, LSHM-CT-2006-037315), the German Research Foundation (SA434/4-2 to T.S.); the German Federal Ministry of Education and Research, National Genome Research Network (NGFNplus: EMINet, 01GS08120 to T.S., 01GS08123 to H.L.). This project received infrastructure support through the PopGen biobank and the DFG excellence cluster “Inflammation at Interfaces.” The KORA research platform (KORA, Cooperative Health Research in the Region of Augsburg) was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, and the Social Ministry of the Federal State of Mecklenburg-West Pomerania. Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 03ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany, and the Federal State of Mecklenburg-West Pomerania. The University of Greifswald is a member of the “Center of Knowledge Interchange” program of the Siemens AG.

## DISCLOSURE

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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## APPENDIX

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** *NRXN1* deletions in controls. Public available CNV data from two independent control cohorts performed by Itsara et al. (2009) and by Shaikh et al. (2009) are depicted for the *NRXN1* region.

**Figure S2.** *NRXN1* deletions in controls. CNV data from a study published by the International Schizophrenia Consortium (International Schizophrenia Consortium, 2008) are depicted for the *NRXN1* region.