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Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies

Carolien G. F. de Kovel,[1,*] Holger Trucks,[2,*] Ingo Helbig,[3,*] Heather C. Mefford,[4,5] Carl Baker,[5] Costin Leu,[2] Christian Kluck,[2] Hiltrud Muhle,[3] Sarah von Spiczak,[3] Philipp Ostertag,[3] Tanja Obermeier,[3] Ailing A. Kleefuß-Lie,[6] Kerstin Hallmann,[6] Michael Steffens,[7] Verena Gaus,[8] Karl M. Klein,[9] Hajo M. Hamer,[9] Felix Rosenow,[9] Eva H. Brilstra,[1] Dorothée Kasteleijn-Nolst Trenité,[1] Marielle E. M. Swinkels,[1] Yvonne G. Weber,[10] Iris Unterberger,[11] Fritz Zimprich,[12] Lydia Urak,[13] Martha Feucht,[13] Karoline Fuchs,[14] Rikke S. Møller,[15,16] Helle Hjalgrim,[15] Peter De Jonghe,[17] Arvid Suls,[17] Ina-Maria Rückert,[18] Heinz-Erich Wichmann,[18,19,20] Andre Franke,[21] Stefan Schreiber,[21] Peter Nürnberg,[2] Christian E. Elger,[6] Holger Lerche,[10] Ulrich Stephani,[3] Bobby P. C. Koeleman,[1] Dick Lindhout,[1,22] Evan E. Eichler[5,23] and Thomas Sander[2,8]

1 Section Complex Genetics, Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands
2 Cologne Centre for Genomics, University of Cologne Cologne, Germany
3 Department of Neuropaediatrics, University Medical Centre Schleswig-Holstein (Kiel Campus), Kiel, Germany
4 Department of Paediatrics, University of Washington, Seattle, USA
5 Department of Genome Sciences, University of Washington, Seattle, USA
6 Department of Epileptology, University of Bonn, Bonn, Germany
7 Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany
8 Department of Neurology, Charité University Medicine, Campus Virchow Clinic, Humboldt University of Berlin, Berlin, Germany
9 Department of Neurology, Interdisciplinary Epilepsy-Centre, Philipps University Marburg, Marburg, Germany
10 Neurological Clinic, University of Ulm, Ulm, Germany
11 University Clinic for Neurology, Medical University of Innsbruck, Innsbruck, Austria
12 Department of Clinical Neurology, Medical University of Vienna, Vienna, Austria
13 Department of Paediatrics and Neonatology, Medical University of Vienna, Vienna, Austria
14 Department for Biochemistry and Molecular Biology, Center for Brain Research, Medical University of Vienna, Vienna, Austria
15 Department of Neurology, Danish Epilepsy Centre, Dianalund, Denmark
16 Wilhelm Johannsen Centre for Functional Genome Research, University of Copenhagen, Copenhagen, Denmark
17 Department of Molecular Genetics, University of Antwerp, Antwerpen, Belgium
18 Institute of Epidemiology, Helmholtz Zentrum München, German Research Centre for Environmental Health, Munich/Neuherberg, Germany
19 Chair of Epidemiology, IBE, University of Munich (LMU), Munich, Germany
20 Klinikum Grosshadern, Munich, Germany
21 Institute for Clinical Molecular Biology, University Medical Centre Schleswig-Holstein (Kiel Campus), Kiel, Germany
22 SEIN Epilepsy Institute in the Netherlands, Heemstede, The Netherlands
23 Howard Hughes Medical Institute, University of Washington, Seattle, USA

*These authors contributed equally to this work.

Correspondence to: Thomas Sander, MD,
Cologne Centre for Genomics,
University of Cologne, Zülpicher Str. 47, 50674 Cologne,
Germany
E-mail: sandert@uni-koeln.de

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Idiopathic generalized epilepsies account for 30% of all epilepsies. Despite a predominant genetic aetiology, the genetic factors predisposing to idiopathic generalized epilepsies remain elusive. Studies of structural genomic variations have revealed a significant excess of recurrent microdeletions at 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11 and 22q11.2 in various neuropsychiatric disorders including autism, intellectual disability and schizophrenia. Microdeletions at 15q13.3 have recently been shown to constitute a strong genetic risk factor for common idiopathic generalized epilepsy syndromes, implicating that other recurrent microdeletions may also be involved in epileptogenesis. This study aimed to investigate the impact of five microdeletions at the genomic hotspot regions 1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2 on the genetic risk to common idiopathic generalized epilepsy syndromes. The candidate microdeletions were assessed by high-density single nucleotide polymorphism arrays in 1234 patients with idiopathic generalized epilepsy from North-western Europe and 3022 controls from the German population. Microdeletions were validated by quantitative polymerase chain reaction and their breakpoints refined by array comparative genomic hybridization. In total, 22 patients with idiopathic generalized epilepsy (1.8%) carried one of the five novel microdeletions compared with nine controls (0.3%) (odds ratio = 6.1; 95% confidence interval 2.8–13.2; $\chi^2 = 26.7$; 1 degree of freedom; $P = 2.4 \times 10^{-7}$). Microdeletions were observed at 1q21.1 [Idiopathic generalized epilepsy (IGE)/control: 1/1], 15q11.2 (IGE/control: 12/6), 16p11.2 IGE/control: 1/0, 16p13.11 (IGE/control: 6/2) and 22q11.2 (IGE/control: 2/0). Significant associations with IGEs were found for the microdeletions at 15q11.2 (odds ratio = 4.9; 95% confidence interval 1.8–13.2; $P = 4.2 \times 10^{-4}$) and 16p13.11 (odds ratio = 7.4; 95% confidence interval 1.3–74.7; $P = 0.009$). Including nine patients with idiopathic generalized epilepsy in this cohort with known 15q13.3 microdeletions (IGE/control: 9/0), parental transmission could be examined in 14 families. While 10 microdeletions were inherited (seven maternal and three paternal transmissions), four microdeletions occurred *de novo* at 15q13.3 ($n = 1$), 16p13.11 ($n = 2$) and 22q11.2 ($n = 1$). Eight of the transmitting parents were clinically unaffected, suggesting that the microdeletion itself is not sufficient to cause the epilepsy phenotype. Although the microdeletions investigated are individually rare (<1%) in patients with idiopathic generalized epilepsy, they collectively seem to account for a significant fraction of the genetic variance in common idiopathic generalized epilepsy syndromes. The present results indicate an involvement of microdeletions at 15q11.2 and 16p13.11 in epileptogenesis and strengthen the evidence that recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 confer a pleiotropic susceptibility effect to a broad range of neuropsychiatric disorders.

Keywords: idiopathic generalized epilepsy; microdeletions; association; genetics

Abbreviations: CNV = copy number variation; IGE = idiopathic generalized epilepsy; SNP = single nucleotide polymorphism

Introduction

The idiopathic generalized epilepsies (IGEs) affect up to 0.3% of the general population and account for 30% of all epilepsies (Jallon and Latour, 2005). The clinical features are characterized by the age-related occurrence of recurrent unprovoked generalized seizures in the absence of detectable brain lesions or metabolic abnormalities (ILAE, 1989). Childhood and juvenile absence epilepsy, juvenile myoclonic epilepsy and epilepsies with generalized tonic–clonic seizures alone represent the most common IGE syndromes (ILAE, 1989). The electroencephalographic signature of IGE seizures is marked by generalized spike–wave discharges, which reflect a synchronized hyperexcitable state of thalamocortical circuits (Blumenfeld, 2005).

Genetic factors play a predominant role in the aetiology of common IGE syndromes. Heritability estimates are >80% and recurrence risk for first-degree relatives varies between 4% and 9% (Helbig *et al*., 2008). Molecular genetic approaches have identified causative gene mutations in mainly rare monogenic forms of idiopathic epilepsies. Most of the currently known genes for human idiopathic epilepsies encode voltage- or ligand-gated ion channels (Reid *et al*., 2009). Despite extensive research, the genetic variants predisposing to common IGE syndromes remain elusive. The genetic architecture is likely to display a biological continuum, in which a small fraction follows monogenic inheritance, whereas the majority of IGE patients presumably display an oligo-/polygenic predisposition.

The role of copy number variations (CNVs) in human disease, and especially in neuropsychiatric disorders, is becoming increasingly evident (Cook and Scherer, 2008; Slavotinek, 2008; Mefford and Eichler, 2009; Sharp, 2009). While many of the observed structural genomic variations have been detected only in individual patients, other CNVs are found recurrently at low frequencies, either *de novo* or inherited (Itsara *et al*., 2009*a*; Mefford and Eichler, 2009; Sharp, 2009). In particular, pathogenic significance of CNVs has been shown for genomic rearrangements flanked by segmental duplications, which promote non-allelic homologous recombinations resulting in recurrent microdeletions or microduplications (Gu *et al*., 2008; Itsara *et al*., 2009*a*). Structural genomic variations in these rearrangement hotspot regions represent many of the genomic disorders identified to date (Slavotinek, 2008; Mefford and Eichler, 2009). It is therefore possible that a bulk of rare CNVs occurring in excess in common disorders collectively explain a substantial fraction of the disease heritability.

A recurrent microdeletion at 15q13.3 was recently shown to constitute a genetic risk factor for common IGE syndromes and was found in 1% of IGE patients whereas it was not detected in controls (Helbig *et al*., 2009). This association was confirmed in an independent IGE sample (Dibbens *et al*., 2009).

This microdeletion was originally described in patients exhibiting mental retardation associated with seizures (Sharp *et al*., 2008), and subsequently in patients with schizophrenia (Schizophrenia Consortium, 2008; Stefansson *et al*., 2008; Kirov *et al*., 2009), psychotic disorder (Miller *et al*., 2009), autism (Miller *et al*., 2009; Pagnamenta et al., 2009) and developmental delay (van Bon *et al*., 2009). The broad phenotypic spectrum associated with the 15q13.3 microdeletion suggests that shared mechanisms might be involved in the pathogenesis of seemingly unrelated neuropsychiatric disorders. Accordingly, the question arises whether additional recurrent microdeletions associated with neuropsychiatric disorders also confer risk to common IGE syndromes.

Five additional large microdeletions at 1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2 are found recurrently in patients either affected by schizophrenia, psychotic disorder, autism or mental retardation (Sebat *et al*., 2007; Ullmann *et al*., 2007; Basset *et al*., 2008; Brunetti-Pierri *et al*., 2008; Cook and Scherer, 2008; Kumar *et al*., 2008; Marshall *et al*., 2008; Mefford *et al*., 2008; Schizophrenia Consortium, 2008; Sharp *et al*., 2009; Slavotinek, 2008; Stefansson *et al*., 2008; Weiss *et al*., 2008; Hannes *et al*., 2009; Itsara *et al*., 2009*b*; Kirov *et al*., 2009; Need *et al*., 2009) and some of the patients reported in the previous studies are also affected by seizures. This association study examined the role of these five recurrent microdeletions in the aetiology of common IGE syndromes.

Subjects and methods

Choice of candidate microdeletions

The selection of microdeletions at 1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2 was based on previous large-scale copy number variation analyses (*1q21.1*: Brunetti-Pierri *et al*., 2008; Mefford *et al*., 2008; Schizophrenia Consortium, 2008; Stefansson *et al*., 2008; Need *et al*., 2009; *15q11.2*: Stefansson *et al*., 2008; Kirov *et al*., 2009; *16p11.2:* Sebat *et al*., 2007; Kumar *et al*., 2008; Marshall *et al*., 2008; Weiss *et al*., 2008; *16p13.11:* Ullmann *et al*., 2007; Hannes *et al*., 2009; Need *et al*., 2009; *22q11.2:* Basset *et al*., 2008; Need *et al*., 2009; Schizophrenia Consortium, 2008) and a recent meta-analysis (Itsara *et al*., 2009*b*) in neuropsychiatric disorders including autism, intellectual disability and schizophrenia (Table 1). The following inclusion criteria for the selection of candidate microdeletions were applied: (i) recurrent non-allelic homologous recombination-generated microdeletion (equal size and defined breakpoints); (ii) previous association of the microdeletion with neuropsychiatric disorders ($P<0.05$) and (iii) size of the deletion larger than >400 kb to ensure a reliable detection by the Affymetrix single nucleotide polymorphism (SNP) 6.0 array (coverage: 200–1500 probe sets). Extending our previous studies (Dibbens *et al*., 2009; Helbig *et al*., 2009), this study focussed on five additional candidate microdeletions at 1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2. To evaluate the relative impact of the six candidate microdeletions on epileptogenesis, nine IGE patients with 15q13.3 deletions observed in this IGE sample were also included in the overall comparison and transmission analysis.

The candidate approach applied in this study tried to avoid the inclusion of a large number of mainly neutral CNVs/CNPs or artificial CNVs detected by a genome-wide CNV scan, which would drastically reduce the power to detect rare pathogenic CNVs.

Study participants

All study participants gave informed consent according to the regulations at their local institutional review boards. Phenotyping and diagnostic classification of IGE syndromes were carried out according to standardized phenotyping protocols available at the Cologne Center for Genomics website (http://www.ccg.uni-koeln.de/epilepsygenetics1.html) (ILAE, 1989). According to the exclusion criteria, individuals with a history of major psychiatric disorders (autism spectrum disorder, schizophrenia and affective disorder) or severe intellectual disability were excluded. In a multi-centre effort, 1234 unrelated IGE patients (458 males, 776 females) were collected from Austria ($n=166$), Belgium ($n=35$), Denmark ($n=72$), Germany ($n=755$) and the Netherlands ($n=206$). The epilepsy sample comprised the following IGE syndromes: childhood/juvenile absence epilepsy ($n=576$); juvenile myoclonic epilepsy ($n=487$), epilepsy with generalized tonic–clonic seizures alone (EGTCS; $n=171$). Notably, 884 of the IGE patients and 1202 of the International Database on the Legal and Socio-ethical Aspects of Population Genetic (PopGen) sector of controls were investigated in a previous study, including eight IGE patients carrying a 15q13.3 deletion (Helbig *et al*., 2009). In addition, 134 IGE patients from the present cohort were part of a replication study (Dibbens *et al*., 2009), but did not carry 15q13.3 deletions.

Affymetrix SNP 6.0 data from 3022 German population controls (1550 males, 1472 females) were obtained from two datasets, the first from the Cooperative Health Research in the Region of Augsburg (KORA: $n=1786$; Wichmann *et al*., 2005) and the second from PopGen ($n=1236$; Krawczak *et al*., 2006). The population controls were not screened for epilepsy or major neuropsychiatric disorders, and consequently a small proportion (<1%) of controls might be affected. All samples were checked for ancestry matching on genotype by EIGENSTRAT analysis (Price *et al*., 2006).

Table 1 Recurrent microdeletions reported in neuropsychiatric disorders

Chrom. segment	Chrom. position (Mb)[a]	MicroDel size (Mb)	Candidate gene	Neuropsychiatric disorder
1q21.1	145.0–146.35	1.35	*GJA5, GJA8, HYDIN2*	ID, SZ
15q11.2	20.3–20.75	0.45	*CYFIP1*	SZ
16p11.2	29.5–30.1	0.7	*KCTD13, SEZ6L2*	ASD, ID
16p13.11	14.7–16.3	1.6	*NDE1*	ID, SZ, ASD
22q11.2	17.5–20.5	3.0	*COMT, SNAP29*	SZ, ID, ASD
15q13.3	28.7–30.3	1.5	*CHRNA7*	ID/EPI, SZ, ASD

a NCBI build 36.
ASD = autism spectrum disorder; EPI = epilepsy; ID = intellectual disability; SZ = schizophrenia.

Genotyping and copy number variation detection

Samples were typed for 1.8 million probe sets on the Affymetrix Genome-Wide Human SNP Array 6.0. The selected microdeletions were covered by 200–1500 probe sets each on the Affymetrix SNP 6.0 array. CNV analysis was performed by the algorithm implemented in the Affymetrix Genotyping Console version 3.0.2. Changes of the heterozygosity state and log2 ratios along with candidate deletions were visually inspected to exclude technical artefacts.

Microdeletions were considered to match the published deletions if they overlapped at least 85% of the genomic region of the candidate microdeletion (Table 1). All deletions identified by Affymetrix SNP 6.0 arrays were verified by real-time quantitative PCR, using a novel Duplex TaqMan CNV assay (Applied Biosystems, TaqMan CN early access program; TaqMan probe sequences are available on request) and/or array comparative genomic hybridization, as described previously (Itsara *et al*., 2009). Array comparative genomic hybridization data were used for refining deletion breakpoints.

Statistical analysis

Association analyses between genotype and phenotype were carried out by two-sided χ^2-tests or Fisher's exact tests where appropriate.

Results

Detection of microdeletions in patients with IGE and controls

Altogether, we detected deletions at the five candidate loci (1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2) in 22 (1.8%) out of 1234 patients and in nine (0.3%) out of 3022 controls [odds ratio (OR) = 6.1; 95% confidence interval (CI) 2.8–13.2; $\chi^2 = 26.7$; 1 degree of freedom; $P = 2.4 \times 10^{-7}$] (Fig. 1, Table 2). Microdeletions at 15q11.2 were observed in 12 (1%) out of 1234 IGE patients representing the most common microdeletion site, but were also observed in six (0.2%) out of 3022 controls (OR = 4.9; 95% CI 1.8–13.2; $\chi^2 = 12.5$; 1df; $P = 4.2 \times 10^{-4}$). In addition, an association with IGE was obtained for 16p13.11 microdeletions, which were found in six (0.5%) out of 1234 IGE patients and two (0.07%) out of 3022 controls (OR = 7.4; 95% CI 1.3–74.7; Fisher's exact test, $P = 0.0094$). Microdeletions at 22q11.2 were observed in two patients and microdeletions at 16p11.2 and 1q21.1 in a single patient each (Fig. 1). A 1q21.1 microdeletion was also identified in one control subject, whereas the other two deletions were not detected in controls. Microdeletions at 15q13.3 were found in nine (0.7%) out of 1234 patients and in none of the 3022 controls (Fisher's exact test, $P = 1.4 \times 10^{-5}$). Eight of the IGE patients with 15q13.3 deletions have been previously reported (Helbig *et al*., 2009) and one patient was identified in the extended IGE sample. Including the 15q13.3 deletions, we detected microdeletions in 31 (2.5%) of 1234 patients and in nine (0.3%) of 3022 controls (OR = 8.6; 95% CI 4.1–18.2; $\chi^2 = 46.1$; 1df; $P = 1.1 \times 10^{-11}$) (Fig. 1, Table 2).

Cosegregation analysis

DNA samples from both parents were available for 14 out of 31 patients with identified microdeletions (Fig. 2). For segregation analysis, all available family members were typed by quantitative PCR and/or array comparative genomic hybridization.

While 10 out of 14 microdeletions were inherited (seven maternal and three paternal transmissions), four *de novo* deletions were identified in 14 IGE patients (Table 3, Fig. 2). DNA from both parents was available for four out of 12 IGE patients carrying a 15q11.2 microdeletion. Maternal inheritance was seen in three and paternal inheritance in one out of four patients. For 16p13.11 microdeletions, DNA samples from both parents were available for four out of six families. *De novo* deletions were observed in two out of four patients and maternal inheritance in two out of four patients. In five out of nine patients with 15q13.3 microdeletions, parental DNA was available. Paternal and maternal inheritances were each found in two out of five transmissions. One *de novo* microdeletion occurred in these five families. Parental DNA was also available for one out of two patients with 22q11.2 microdeletions, in whom a *de novo* deletion was observed.

Deletions in 15 IGE probands were shared with five affected and 14 unaffected first-degree relatives, while four first-degree relatives affected with IGE did not carry the deletion (Fig. 2). These four affected family members without a deletion were all found in families exhibiting the 15q11.2 deletion. In 10 out of 15 families, the microdeletions were inherited (three paternally and seven maternally). Eight of the transmitting parents (15q11.2: $n = 3$; 15q13.3: $n = 3$; 16p13.11: $n = 2$) were clinically unaffected, one father carrying a 15q13.3 deletion was affected by IGE and one mother with a 15q11.2 deletion had a history of febrile seizures (Fig. 2). Cosegregation between the microdeletions and the phenotype was not consistent with autosomal dominant inheritance, particularly in three large families with 15q11.2 microdeletions (Fig. 2; families: F50, F157 and F9831).

Genotype–phenotype correlations

The deletions investigated in this study are flanked by highly homologous segmental duplications and non-allelic homologous recombination is thought to promote genomic rearrangements between the putative breakpoints (Hastings *et al*., 2009; Itsara *et al*., 2009*a*; Sharp, 2009). The exact positions of the deletion breakpoints inside the segmental duplication clusters are difficult to determine and results may vary between array platforms depending on the genomic coverage of the array probe sets across the flanking segmental duplications. We designed a customized oligonucleotide microarray to refine further the breakpoints of the microdeletions characterized in this study (Itsara *et al*., 2009*a*). The individual breakpoint estimates and the resulting sizes of the microdeletions are shown in Table 3, Fig. 1 and Supplementary Fig. S1. The individual breakpoints and sizes of the observed microdeletions consistently corresponded with those described previously (Table 1).

Patients with the different deletions ($n = 31$) displayed a representative distribution of IGE syndromes (childhood absence epilepsy/juvenile absence epilepsy 48.4%, juvenile myoclonic

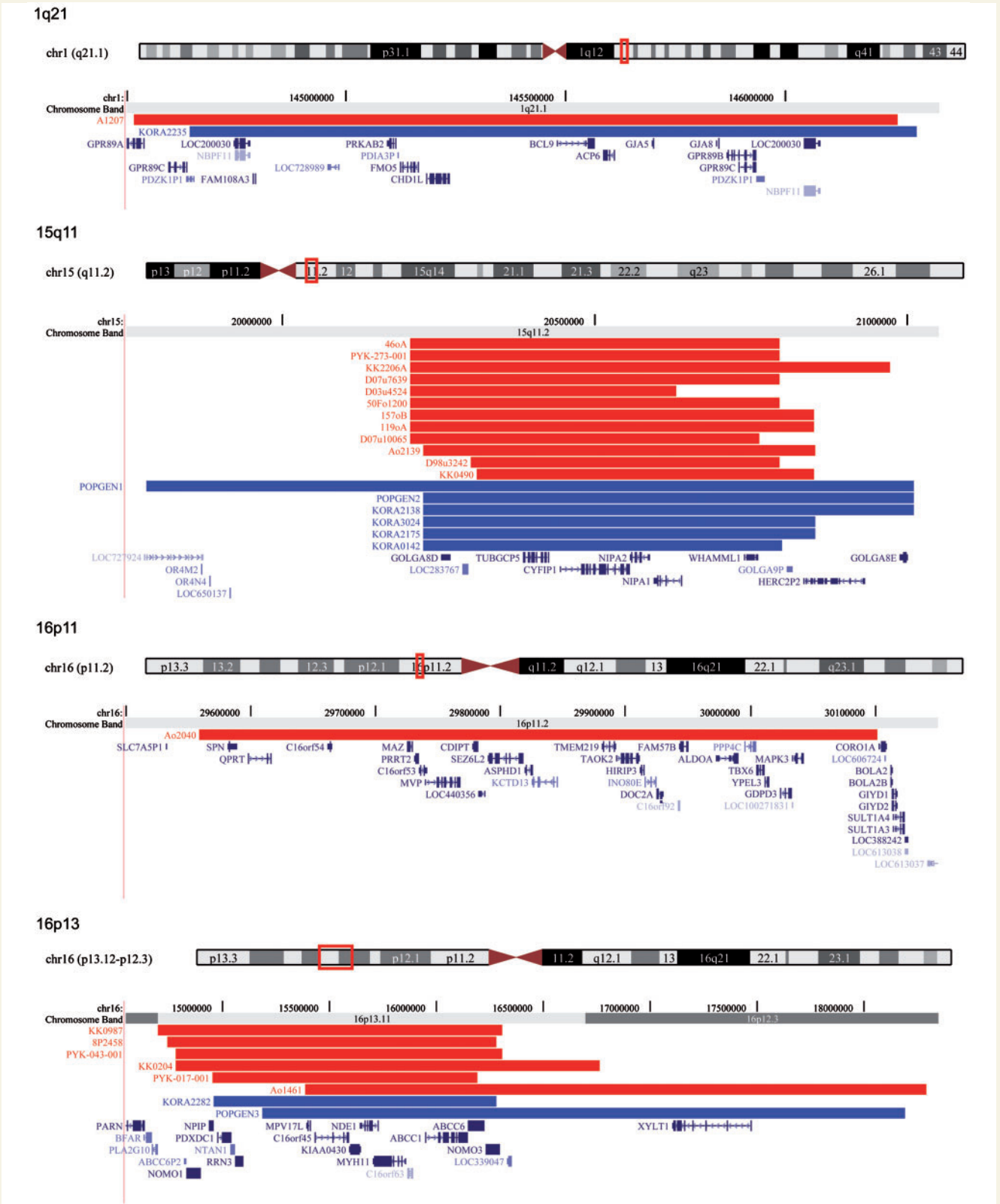

Figure 1 Genomic position of the microdeletions at the genomic hot spot regions 1q21.1, 15q11.2, 16p11.2, 16p13.11, 22q11.2 and 15q13.3. Red = IGE patients; blue = controls. The positions of genes are also shown. Produced with the University of California, Santa Cruz Genome Browser (http://www.genome.ucsc.edu).

(Continued)

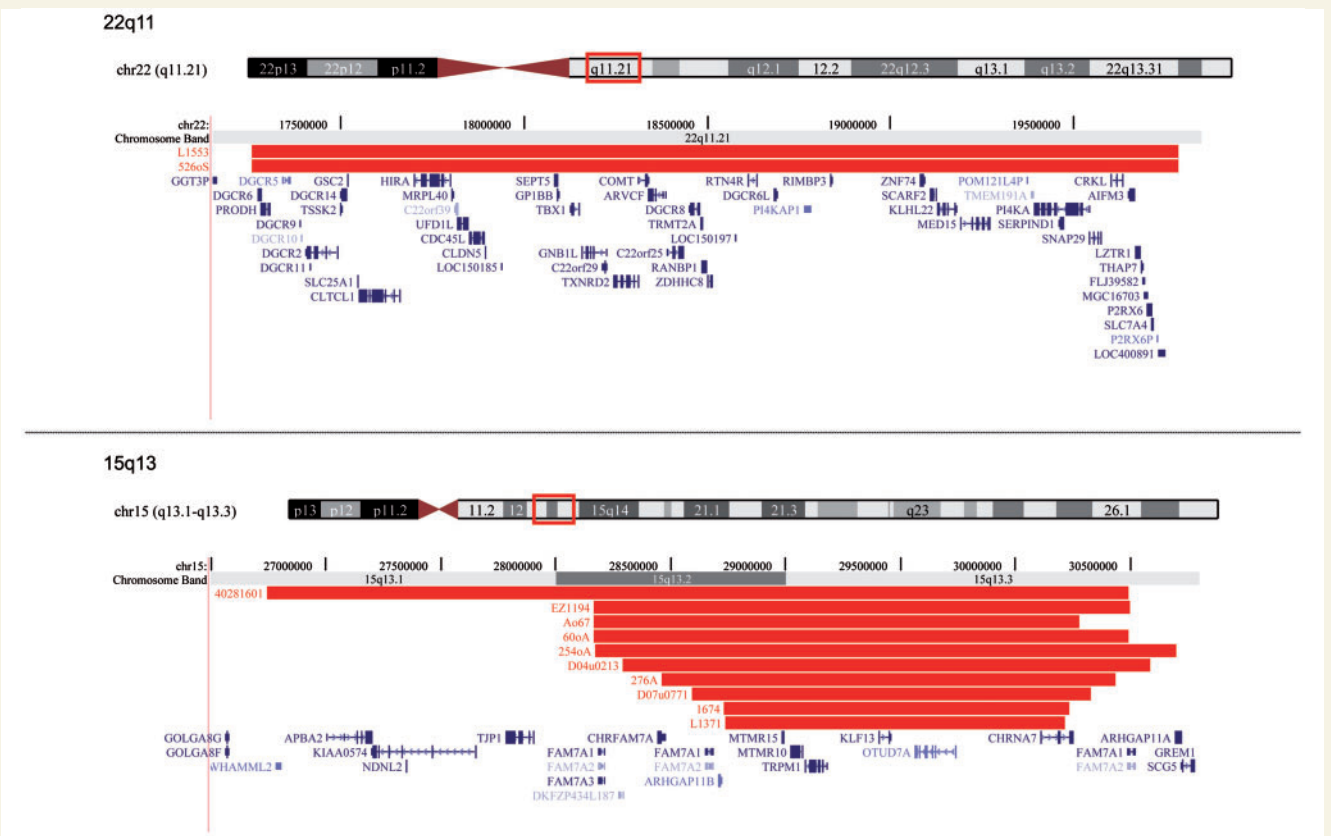

Figure 1 Continued.

Table 2 Recurrent microdeletions in IGE patients and controls

Chromosome region	IGE $n=1234$	Controls $n=3022$	*P*-value two-sided**	USA sample $n=2493$[a]
1q21.1	1	1	–	0
15q11.2	12	6	**4.2×10^{-4}**	4
16p11.2	1	0	–	0
16p13.11	6	2	**0.0094***	0
22q11.2	2	0	–	0
Microdels w/o 15q13.3	**22**	**9**	**2.4×10^{-7}**	4
15q13.3	9	0	**1.4×10^{-5}***	0
Microdels total	**31**	**9**	**1.1×10^{-11}**	4

a Samples of 1607 USA subjects of European ancestry and 886 subjects from the Human Genome Diversity Panel (Itsara *et al*., 2009*a*).
*Fisher's exact test; **significant *P*-values.
Bold values indicate statistical significance.

epilepsy 35.5%, epilepsy with generalized tonicclonic seizures 16.1%; males 29%, females 71%) similar to that observed in the entire IGE sample (childhood absence epilepsy/juvenile absence epilepsy 46.6%, juvenile myoclonic epilepsy 39.5%, epilepsy with generalized tonic–clonic seizures 13.9%; males 37%, females 63%). We found no evidence that patients with microdeletions had refractory seizures and there was no preponderance of a particular seizure type or a shift towards an early age of onset (Table 3). We did not observe other neuropsychiatric phenotypes in family members carrying a deletion.

Discussion

In the present study, we investigated whether five large recurrent microdeletions (at 1q21.1, 15q11.2, 16p11.2, 16p13.11 and 22q11.2), previously associated with neuropsychiatric disorders, also confer risk to common IGE syndromes. Recurrent microdeletions were found at 15q11.2 ($n=12$), 16p13.11 ($n=6$) and 22q11.2 ($n=2$) in 1234 IGE patients. The microdeletions at 1q21.1 and 16p11.2 occurred in one IGE patient each. Altogether, the five microdeletions showed a significant excess in the IGE patients compared with controls ($P=2.4 \times 10^{-7}$) and the present association results indicate an involvement of microdeletions at 15q11.2 and 16p13.11 in the aetiology of IGE (Table 2). Including the 15q13.3 deletions (IGE/control: 9/0), recurrent microdeletions were present in 2.5% of 1234 IGE patients versus 0.3% of 3022 population controls ($P=1.1 \times 10^{-11}$). IGE patients carrying a microdeletion display typical clinical features of IGE regarding seizure types and age of onset. Although the microdeletions investigated are individually rare (<1%) in patients with IGE, they collectively account for a significant fraction of the genetic variance of common IGE syndromes.

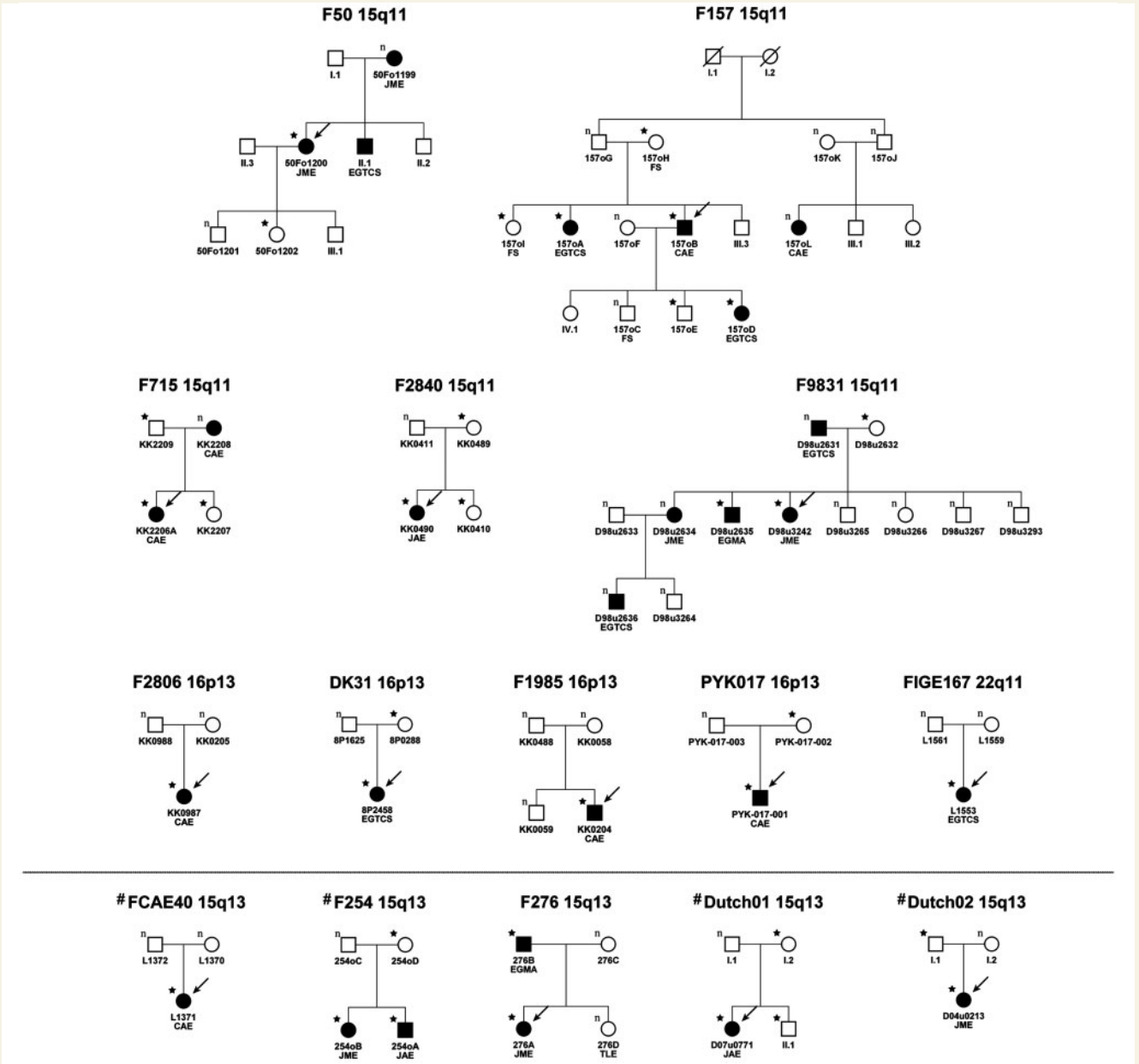

Figure 2 Familial segregation of the microdeletions at 15q11.2, 16p13.11, 22q11.2 and 15q13.3. Arrows denote the index-IGE patient typed by the Affymetrix SNP 6.0 array. Black symbols = individuals affected by IGE. FS = febrile seizure; CAE = childhood absence epilepsy; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; EGTCS = epilepsy with generalized tonic–clonic seizures alone; EGMA = epilepsy with generalized tonic–clonic seizures on awakening; TLE = temporal lobe epilepsy; copy number state: n = normal/two copies; filled star indicates deletion carrier. #Families of IGE patients with 15q13.3 deletions reported previously (Helbig *et al*., 2009).

The investigated microdeletions seem to differ with regard to the magnitude of the epileptogenic effect (e.g. point estimates of odds ratio), occurrence of *de novo* deletions and familial segregation patterns (Tables 2 and 3, Fig. 2). The 15q13.3 microdeletion emerged as the major genetic risk factor with a point estimate of OR > 50 (95% CI 21.7–139.6), assuming a frequency <0.02% in the general population (Schizophrenia Consortium, 2008; Sharp *et al*., 2008; Stefansson *et al*., 2008; Dibbens *et al*., 2009; Helbig *et al*., 2009; Kirov *et al*., 2009). In contrast, 15q11.2 (OR = 4.9) and 16p13.11 (OR = 7.4) microdeletions seem to confer a lower genetic risk to IGE, also reflected by the higher frequency in controls (0.20% and 0.07%, respectively).

Segregation between microdeletions and the IGE trait was investigated if family members were available for testing. Particularly, the 15q11.2 microdeletion did not cosegregate with the IGE trait in three large families (Fig. 2). For the other deletions,

Table 3 Characteristics of study participants carrying a candidate deletion

Sample ID	Sex	Chrom.	Start	End	Size (kb)	Array	Age-at-onset (years)	Seizure types	IGE syndrome	Intellectual status	Inheritance
A1207	F	1q21.1	144 516 731	146 256 137	1 739	Array CGH	23	abs., GTCS	JAE	Normal	Unknown
46oA	M	15q11.2	20 203 685	20 795 813	592	Array CGH	15	abs., myocl., GTCS	JME	Normal	Unknown
D07u7639	F	15q11.2	20 203 685	20 795 813	592	Array CGH	14	myocl., GTCS	JME	Normal	Unknown
KK2206A	F	15q11.2	20 203 685	20 971 169	767	Array CGH	4	abs.	CAE	Normal	Paternal
PYK-273-001	F	15q11.2	20 203 685	20 795 813	592	Array CGH	5	abs.	CAE	Normal	Unknown
119oA	F	15q11.2	20 204 077	20 850 488	646	Array CGH	12	GTCS	EGTCS	Normal	Unknown
157oB	M	15q11.2	20 204 077	20 850 488	646	Array CGH	4	abs.	CAE	Normal	Parental
50Fo1200	F	15q11.2	20 204 077	20 795 813	592	Array CGH	Puberty	myocl.	JME	Normal	Maternal
D03u4524	M	15q11.2	20 204 077	20 629 367	425	Array CGH	16	abs., myocl.	JME	Normal	Unknown
D07u10065	F	15q11.2	20 204 077	20 763 914	560	Array CGH	10	abs., GTCS	JAE	Normal	Unknown
Ao2139	F	15q11.2	20 224 751	20 852 202	1 485	Affy 6.0 array	7	abs., myocl.	JME	Normal, legasthenia	Unknown
D98u3242	F	15q11.2	20 301 665	20 795 813	494	Array CGH	4	abs., myocl., GTCS	JME	Normal	Maternal
KK0490	F	15q11.2	20 310 606	20 850 488	540	Array CGH	15	abs., GTCS	JAE	Normal	Maternal
Ao2040	F	16p11.2	29 559 251	30 101 408	542	Array CGH	<20	GTCS	EGTCS	Normal	Unknown
8P2458	F	16p13.11	14.742.556	16.285.151	1.543	Affy 6.0 array	9	GTCS	EGTCS	Normal	Maternal
KK0987	F	16p13.11	14 699 106	16 308 654	1 610	Array CGH	Childhood	abs., FS	CAE	Learning disability	*De novo*
PYK-043-001	F	16p13.11	14 785 031	16 308 654	1 524	Array CGH	5	abs.	CAE	Normal	Unknown
KK0204	M	16p13.11	14 785 031	16 767 009	1 982	Array CGH	8	abs., GTCS	CAE	Normal	*De novo*
PYK-017-001	M	16p13.11	14 956 201	16 193 208	1 237	Array CGH	7	abs.	CAE	Normal	Maternal
Ao1461	F	16p13.11	15 386 338	18 291 982	2 906	Array CGH	17	myocl., GTCS	JME	Learning disability	Unknown
526oS	M	22q11.2	17 258 339	19 786 713	2 528	Array CGH	9	GTCS	EGTCS	Minor developmental delay	Unknown
L1553	F	22q11.2	17 258 339	19 786 713	2 528	Array CGH	20	GTCS	EGTCS	Normal	*De novo*
D07u0771[a]	F	15q13.3	28.595.222	30.326.817	1.732	Affy 6.0 array	14	abs.	JAE	Normal	Maternal
40281601[a]	F	15q13.3	26 745 821	30 494 518	3 749	Array CGH	3	abs., GTCS	CAE	Normal	Unknown
60oA[a]	M	15q13.3	28 168 397	30 491 740	2 323	Array CGH	12	abs., GTCS	JAE	Normal	Unknown
Ao67[a]	F	15q13.3	28 168 397	30 276 525	2 108	Array CGH	16	myocl., GTCS	JME	Normal	Unknown
EZ1194[a]	M	15q13.3	28 168 397	30 498 257	2 330	Array CGH	6	abs., myocl., GTCS	JME	Normal	Unknown
254oA[a]	M	15q13.3	28 171 483	30 701 463	2 530	Array CGH	9	abs., GTCS	JAE	Normal	Maternal
276A	F	15q13.3	28 461 375	30 436 131	1 975	Array CGH	14	myocl.	JME	Normal	Paternal
L1371[a]	F	15q13.3	28 738 025	30 215 571	1 478	Array CGH	4	abs.	CAE	Minor developmental delay	*De novo*
KORA2235	M	1q21.1	144 643 813	146 297 795	1 654	Affy 6.0 array	–	–	Control	Unknown	Unknown
POPGEN1	M	15q11.2	19 781 829	21 010 631	1 229	Affy 6.0 array	–	–	Control	Unknown	Unknown
KORA0142	F	15q11.2	20 224 751	20 799 862	575	Affy 6.0 array	–	–	Control	Unknown	Unknown
KORA2138	F	15q11.2	20 224 751	21 010 631	786	Affy 6.0 array	–	–	Control	Unknown	Unknown
KORA2175	F	15q11.2	20 224 751	20 852 202	627	Affy 6.0 array	–	–	Control	Unknown	Unknown
KORA3024	M	15q11.2	20 224 751	20 852 202	627	Affy 6.0 array	–	–	Control	Unknown	Unknown
POPGEN2	M	15q11.2	20 224 751	21 010 631	786	Affy 6.0 array	–	–	Control	Unknown	Unknown
KORA2282	F	16p13.11	14 961 214	16 285 151	1 324	Affy 6.0 array	–	–	Control	Unknown	Unknown
POPGEN3	M	16p13.11	15 186 307	18 192 575	3 006	Affy 6.0 array	–	–	Control	Unknown	Unknown

a Patients carrying a 15q13.3 deletion reported previously (Helbig *et al*., 2009).

comparative genomic hybridization (CGH), abs. = absence seizures; myocl. = bilateral myoclonic seizures on awakening; GTCS = generalized tonic–clonic seizures; FS = febrile seizures; CAE = childhood absence epilepsy; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; EGTCS = epilepsy with generalized tonic–clonic seizures; F = female; *M* = male.

large families were not available for segregation analysis. Consistent with two small families in which 15q13.3 deletions segregated with affected family members in the present study, Dibbens and colleagues (2009) found incomplete penetrance of the 15q13.3 microdeletion in four out of seven pedigrees and three pedigrees included family members with IGE lacking the 15q13.3 deletion. Despite the remarkable odds ratio (OR > 50), 15q13.3 deletions are not sufficient to express a disease phenotype, which might also vary considerably depending on the genetic background and possible environmental effects. *De novo* microdeletions at 15q13.3, 16p13.11 and 22q11.2 were observed in four out of 14 families, for which DNA was available from both parents. The presence of *de novo* deletion events in conjunction with low population frequencies implicates purifying selection and thus may suggest a strong influence on the disease phenotype.

Taking into account all published studies, remarkable phenotypic variability is observed for carriers of the six recurrent microdeletions assessed in our study, ranging from apparently unaffected carriers to individuals with severe cognitive deficits, dysmorphisms and various neuropsychiatric features. The present epilepsy sample was ascertained by the IGE phenotype excluding those patients affected by major psychiatric and mental disorders. Moreover, carriers of microdeletions were re-evaluated for the presence of intellectual disability or other neuropsychiatric disorders. It is therefore unlikely that the excess of microdeletions found in our study is caused by unobserved comorbidity of neuropsychiatric disorders and IGE.

The mechanisms by which microdeletions mediate their pathogenic effects remain unknown (Itsara *et al*., 2009; Sharp, 2009). Haploinsufficiency of the deleted segment seems the most likely mechanism (Itsara *et al*., 2009*a*; Sharp, 2009) and several plausible candidate genes have been suggested (Table 1). Besides purely stochastic or environmental effects, other genetic mechanisms such as imprinting, unmasking of different recessive allelic mutations on the intact homologous chromosomal segment and background genomic variation may contribute to the highly variable phenotypic expression (Sharp, 2009).

Overall, emerging evidence suggests that recurrent microdeletions may confer a pleiotropic effect underlying various neuropsychiatric disorders. The complex interaction with additional factors might determine the specific phenotype. For example, the 16p13.11 candidate gene *NDE1* (encoding NudE nuclear distribution gene E homologue 1) is known to interact with *DISC1* (gene disrupted in schizophrenia) and *LIS1* (gene causing lissencephaly 1, *PAFAH1B1*). Deficiency of the LIS1–NDE1 complex impairs cortical neurogenesis and neuronal migration (Pawlisz *et al*., 2008) frequently leading to epilepsy, whereas DISC1-NDE1 deficiency appears to play a role in neuropsychiatric disorders, including schizophrenia and bipolar affective disorder (Hennah *et al*., 2009). Together, our findings support the role of neurodevelopmental processes in epileptogenesis. Given the frequency of recurrent microdeletions in various neuropsychiatric and neurodevelopmental disorders, identification of genetic and non-genetic factors determining phenotype specificity will be a major focus of future research.

Despite the high heritability of IGE, the genetic architecture remains elusive. Relatively few epilepsy genes have been identified thus far, mainly in rare monogenic forms of idiopathic epilepsies (Helbig *et al*., 2008; Reid *et al*., 2009). By identifying recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 as collectively significant genetic risk factors for IGE, our study provides new insights into the complex genetic predisposition of common epilepsies. Although the risk estimate of microdeletions associated with IGE is considerably higher (OR: 5–50) than that observed for common SNPs in complex traits (OR < 2), it is much lower than that of highly penetrant mutations causing Mendelian diseases (OR > 100). Our present family study revealed a high percentage (>70%) of apparently unaffected parents transmitting the microdeletion to the affected child (Fig. 2), suggesting that the microdeletion alone is not sufficient to cause an epilepsy phenotype in some cases. Likewise, unprecedented phenotypic heterogeneity has been found for seemingly identical microdeletions at 1q21.1, 16p11.2, 15q13.3 and 22q11.2, ranging from severe genomic syndromes (e.g. 22q11.2 microdeletion: DiGeorge syndrome, velocardiofacial syndrome) to a wide range of neuropsychiatric disorders (e.g. schizophrenia, intellectual disability and autism spectrum disorder), as well as in apparently unaffected individuals (for review see Mefford and Eichler, 2009). With regard to the highly variable phenotypic expressivity of the microdeletions investigated, it is difficult to assess the clinical relevance and implications for genetic counselling, and further studies are clearly needed to specify the phenotype–genotype relationship. Advances in large-scale sequencing and high-resolution mapping of structural genomic rearrangements will provide a survey on structural genomic variations at the genome-wide level, allowing for a more comprehensive assessment of the impact of structural genomic variations in common seizure disorders in the near future (Itsara *et al*., 2009*a*; Mefford and Eichler, 2009).

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Supplementary material

Supplementary material is available at *Brain* online.

References

Basset AS, Marshall CR, Lionel AC, Chow EWC, Scherer SW. Copy number variations and risk for schizophrenia in 22q11.2 deletion syndrome. Hum Mol Genet 2008; 17: 4045–53.

Blumenfeld H. Cellular and network mechanisms of spike-wave seizures. Epilepsia 2005; 46 (Suppl 9): 21–33.

Brunetti-Pierri N, Berg JS, Scaglia F, Belmont J, Bacino CA, Sahoo T, et al. Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. Nat Genet 2008; 40: 1466–71.

Cook EH, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. Nature 2008; 455: 919–23.

Dibbens LM, Mullen S, Helbig I, Mefford HC, Bayly MA, Bellows S, et al. Familial and sporadic 15q13.3 microdeletions in idiopathic generalized epilepsy: precedent for disorders with complex inheritance. Hum Mol Genet 2009; 18: 3626–31.

Gu W, Zhang F, Lupski JR. Mechanisms for human genomic rearrangements. PathoGenetics 2008; 1: 4.

Hannes FD, Sharp AJ, Mefford HC, de Ravel T, Ruivenkamp CA, Breuning MH, et al. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. J Med Genet 2009; 46: 223–32.

Hastings PJ, Lupski JR, Rosenberg SM, Ira G. Mechanisms of change in gene copy number. Nat Rev Genet 2009; 10: 551–64.

Helbig I, Scheffer IE, Mulley JC, Berkovic SF. Navigating the channels and beyond: unravelling the genetics of the epilepsies. Lancet Neurol 2008; 7: 231–45.

Helbig I, Mefford HC, Sharp AJ, Guipponi M, Fichera M, Franke A, et al. 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. Nat Genet 2009; 41: 160–2.

Hennah W, Porteous D. The DISC1 pathway modulates expression of neurodevelopmental, synaptogenic and sensory perception genes. PLoS One 2009; 4: e4906.

International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes: Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 1989; 30: 389–99.

Itsara A, Cooper GM, Baker C, Girirajan S, Li J, Absher D, et al. Population analysis of large copy number variants and hotspots of human genetic disease. Am J Hum Genet 2009a; 84: 148–61.

Itsara A, Cooper GM, Baker C, Girirajan S, Li J, Absher D, et al. Population analysis of large copy number variants and hotspots of human genetic disease. Addendum. Am J Hum Genet 2009b; 84: 550–1.

Jallon P, Latour P. Epidemiology of idiopathic generalized epilepsies. Epilepsia 2005; 46 (Suppl 9): 10–4.

Kirov G, Grozeva D, Norton N, Ivanov D, Mantripragada KK, Holmans P, et al. Support for the involvement of large copy number variants in the pathogenesis of schizophrenia. Hum Mol Genet 2009; 18: 1497–503.

Krawczak M, Nikolaus S, von Eberstein H, Croucher PJ, El Mokhtari NE, Schreiber S. PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. Community Genet 2006; 9: 55–61.

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, et al. Recurrent 16p11.2 microdeletions in autism. Hum Mol Genet 2008; 17: 628–38.

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, et al. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 2008; 82: 477–88.

Mefford HC, Eichler EE. Duplication hotspots, rare genomic disorders, and common disease. Curr Opin Genet Dev 2009; 19: 196–204.

Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, Buysse K, et al. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. N Engl J Med 2008; 359: 1685–99.

Miller DT, Shen Y, Weiss LA, Korn J, Anselm I, Bridgemohan C, et al. Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatirc disorders. J Med Genet 2009; 46: 242–8.

Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, et al. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet 2009; 5: e1000373.

Pagnamenta AT, Wing K, Akha ES, Knight SJ, Bolte S, Schmotzer G, et al. A 15q13.3 microdeletion segregating with autism. Eur J Hum Genet 2009; 17: 687–92.

Pawlisz AS, Mutch C, Wynshaw-Boris A, Chenn A, Walsh CA, Feng Y. Lis1-Nde1-dependent neuronal fate control determines cerebral cortical size and lamination. Hum Mol Genet 2008; 17: 2441–55.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006; 38: 904–9.

Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. Prog Neurobiol 2009; 87: 41–57.

Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 2008; 455: 237–41.

Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, et al. Strong association of de novo copy number mutations with autism. Science 2007; 316: 445–9.

Sharp AJ. Emerging themes and new challenges in defining the role of structural variation in human disease. Hum Mutat 2009; 30: 135–44.

Sharp AJ, Mefford HC, Li K, Baker C, Skinner C, Stevenson RE, et al. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. Nat Genet 2008; 40: 322–8.

Slavotinek AM. Novel microdeletion syndromes detected by chromosome microarrays. Hum Genet 2008; 124: 1–17.

Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, et al. Large recurrent microdeletions associated with schizophrenia. Nature 2008; 455: 232–6.

Ullmann R, Turner G, Kirchhoff M, Chen W, Tonge B, Rosenberg C, et al. Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. Hum Mutat 2007; 28: 674–82.

van Bon BW, Mefford HC, Menten B, Koolen DA, Sharp AJ, Nillesen WM, et al. Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. J Med Genet 2009; 46: 511–23.

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, et al. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 2008; 358: 667–75.

Wichmann HE, Gieger C, Illig T, MONICA/KORA Study Group. KORA-gen—resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 2005; 67 (Suppl 1): S26–30.