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 Dorothee Kasteleijn-Nolst Trenité,1 Marielle E. M. Swinkels,1 Yvonne G. Weber,10 Iris Unterberger,11 Fritz Zimprich,12 Lydia Uruk,13 Martha Feucht,13 Karoline Fuchs,14 Rikke S. Møller,15,16 Helle Hjalgrim,15 Peter De Jonghe,17 Arvid Suls,17 Ina-Maria Rücker,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. Eichler5,23 and Thomas Sander2,8

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, Philippus 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 of 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, 18E, 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


© The Author (2009). Published by Oxford University Press on behalf of the Guarantors of Brain. All rights reserved.

For Permissions, please email: journals.permissions@oxfordjournals.org
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., 2009a; 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., 2009a). 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; Pagamenta 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., 2009b; 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., 2009b) 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 microdeletion 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

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

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 χ2-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; χ2 = 26.7; 1 degree of freedom; P = 2.4 × 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; χ2 = 12.5; 1df; P = 4.2 × 10−5). 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 = 4.9; 95% CI 1.8–13.2; χ2 = 5.1; 1df; P = 0.024). Microdeletions at 22q11.2 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; χ2 = 12.5; 1df; P = 4.2 × 10−5). 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 = 4.9; 95% CI 1.8–13.2; χ2 = 5.1; 1df; P = 0.024). Microdeletions at 22q11.2 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; χ2 = 12.5; 1df; P = 4.2 × 10−5). 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 = 4.9; 95% CI 1.8–13.2; χ2 = 5.1; 1df; P = 0.024). Microdeletions at 22q11.2 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; χ2 = 12.5; 1df; P = 4.2 × 10−5). 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 = 4.9; 95% CI 1.8–13.2; χ2 = 5.1; 1df; P = 0.024).

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., 2009a; 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., 2009a). 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, 16p11.12, 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).
epilepsy 35.5%, epilepsy with generalized tonic–clonic 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 × 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 × 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.

### Table 2 Recurrent microdeletions in IGE patients and controls

<table>
<thead>
<tr>
<th>Chromosome region</th>
<th>IGE n = 1234</th>
<th>Controls n = 3022</th>
<th>P-value two-sided</th>
<th>USA sample n = 2493</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q21.1</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>15q11.2</td>
<td>12</td>
<td>6</td>
<td>4.2 × 10⁻⁴</td>
<td>4</td>
</tr>
<tr>
<td>16p11.2</td>
<td>1</td>
<td>0</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>16p13.11</td>
<td>6</td>
<td>2</td>
<td>0.0094*</td>
<td>0</td>
</tr>
<tr>
<td>22q11.2</td>
<td>2</td>
<td>0</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Microdels w/o 15q13.3</td>
<td>22</td>
<td>9</td>
<td>2.4 × 10⁻⁷</td>
<td>4</td>
</tr>
<tr>
<td>15q13.3</td>
<td>9</td>
<td>0</td>
<td>1.4 × 10⁻⁵*</td>
<td>0</td>
</tr>
<tr>
<td>Microdels total</td>
<td>31</td>
<td>9</td>
<td>1.1 × 10⁻¹¹</td>
<td>4</td>
</tr>
</tbody>
</table>

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

epilepsy 35.5%, epilepsy with generalized tonic–clonic 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.
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 = 4.50 (95% CI 21.7–139.6), assuming a frequency of 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>
<thead>
<tr>
<th>Sample ID</th>
<th>Sex</th>
<th>Chrom.</th>
<th>Start</th>
<th>End</th>
<th>Size (kb)</th>
<th>Array</th>
<th>Age-at-onset (years)</th>
<th>Seizure types</th>
<th>IGE syndrome</th>
<th>Intellectual status</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1207</td>
<td>F</td>
<td>1q21.1</td>
<td>144516731</td>
<td>146256137</td>
<td>1739</td>
<td>Array CGH</td>
<td>23</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>46oA</td>
<td>M</td>
<td>15q11.2</td>
<td>20203685</td>
<td>20795813</td>
<td>592</td>
<td>Array CGH</td>
<td>15</td>
<td>abs., myocl., GTCS</td>
<td>JME</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u4739</td>
<td>F</td>
<td>1q12.2</td>
<td>20203685</td>
<td>20795813</td>
<td>592</td>
<td>Array CGH</td>
<td>14</td>
<td>myocl., GTCS</td>
<td>JME</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>KK2206A</td>
<td>F</td>
<td>15q11.2</td>
<td>20203685</td>
<td>20795813</td>
<td>592</td>
<td>Array CGH</td>
<td>4</td>
<td>abs.</td>
<td>CAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>PYK-273-001</td>
<td>F</td>
<td>15q11.2</td>
<td>20203685</td>
<td>20795813</td>
<td>592</td>
<td>Array CGH</td>
<td>5</td>
<td>abs.</td>
<td>CAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>119oA</td>
<td>F</td>
<td>20140477</td>
<td>20850488</td>
<td>646</td>
<td>Array CGH</td>
<td>12</td>
<td>GTCS</td>
<td>EGTCS</td>
<td>Normal</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>157oB</td>
<td>M</td>
<td>15q11.2</td>
<td>20203685</td>
<td>20795813</td>
<td>592</td>
<td>Array CGH</td>
<td>4</td>
<td>abs.</td>
<td>CAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>50F1200</td>
<td>M</td>
<td>15q11.2</td>
<td>20203685</td>
<td>20795813</td>
<td>592</td>
<td>Array CGH</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>7</td>
<td>abs., myocl.</td>
<td>JME</td>
<td>Normal, legasthenia</td>
<td>Unknown</td>
</tr>
<tr>
<td>D05u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>D07u10065</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ao2139</td>
<td>F</td>
<td>15q11.2</td>
<td>20204077</td>
<td>20852022</td>
<td>1485</td>
<td>Affy 6.0 array</td>
<td>10</td>
<td>abs., GTCS</td>
<td>JAE</td>
<td>Normal</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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., 2009a; 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; Mefford and Eichler, 2009).

Acknowledgements

We thank all individuals and their families for participating in this study.

Funding

European Community (FP6 Integrated Project EPICURE, LSHM-CT-2006-037315, FP6 MEXT visual sensitivity, grant no. 024224 to D.K.-N.T.); the German Research Foundation (SA434/4-2 to T.S. and P.N.); the German Federal Ministry of Education and Research, National Genome Research Network (NGFN-2: NeuroNet to E.C.E., T.S. and H.L.; NGFNplus: EMNet, 01GS08120 to P.N and T.S. and 01GS08123 to H.L.); the Belgian National Fund for Scientific Research (Flanders, 0399.08 to P.J.); The Netherlands National Epilepsy Fund (grant no. 04-08 to B.P.C.K. and D.L.); The Netherlands Organization for Scientific Research (grant no. 917.66.315 to B.P.C.K. and C.G.F.d.K.); and the National Institutes of Health (HD043569 to E.E.E., HD043376 to B.P.C.K. and D.L.); The Netherlands Organization for Scientific Research (grant no. LSHM-CT-2006-037315, FP6 MEXT visual sensitivity, grant no. 024224 to D.K.-N.T.); the German Research Foundation (SA434/4-2 to T.S. and P.N.); the German Federal Ministry of Education and Research, National Genome Research Network (NGFN-2: NeuroNet to E.C.E., T.S. and H.L.; NGFNplus: EMNet, 01GS08120 to P.N and T.S. and 01GS08123 to H.L.); the Belgian National Fund for Scientific Research (Flanders, 0399.08 to P.J.); The Netherlands National Epilepsy Fund (grant no. 04-08 to B.P.C.K. and D.L.); The Netherlands Organization for Scientific Research (grant no. 917.66.315 to B.P.C.K. and C.G.F.d.K.); and the National Institutes of Health (HD043569 to E.E.E., HD043376 to H.C.M., partial); the PopGen biobank (to A.F. and S.S.). The PopGen project received infrastructure support through the German Research Foundation excellence cluster ‘Inflammation at Interfaces’. The KORA research platform (KORA, Cooperative Research in the Region of Augsburg) was initiated and financed by the Helmholtz Zentrum München—German Research Centre.
for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria.

Supplementary material

Supplementary material is available at Brain online.

References


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.


