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15q13.3 microdeletions increase risk of idiopathic generalized epilepsy

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We identified 15q13.3 microdeletions encompassing the *CHRNA7* gene in 12 of 1,223 individuals with idiopathic generalized epilepsy (IGE), which were not detected in 3,699 controls (joint $P = 5.32 \times 10^{-8}$). Most deletion carriers showed common IGE syndromes without other features previously associated with 15q13.3 microdeletions, such as intellectual disability, autism or schizophrenia. Our results indicate that 15q13.3 microdeletions constitute the most prevalent risk factor for common epilepsies identified to date.

Idiopathic generalized epilepsies (IGE) are common seizure disorders accounting for up to one-third of all epilepsies[1]. The vast majority of individuals with IGE have a complex genetic etiology[2], for which the underlying genetic alterations remain largely unknown. Recently, a 15q13.3 microdeletion syndrome has been identified in 0.2–0.3% of individuals with mental retardation and epilepsy[3], schizophrenia[4,5], autism and other neuropsychiatric features[6]. The critical region of the 1.5-Mb deletion on 15q13.3 contains at least seven genes, including the *CHRNA7* gene coding for the α7 subunit of the nicotinergic acetylcholine receptor, which is considered a plausible candidate gene for the epilepsy phenotype.

Susceptibility loci for common idiopathic epilepsies, comprising benign epilepsy of childhood with centrotemporal spikes[7] and common IGE syndromes[8,9], have also been mapped to the 15q13–q14 region. To test whether the 15q13.3 deletion increases risk of common epilepsies, we screened for structural variants within the 15q13.3 region in two independent samples of individuals with IGE and ancestrally matched controls. The first sample comprised 647 unrelated IGE cases of Western European ancestry (EPICURE sample) and 1,202 German controls (PopGen) genotyped using the Affymetrix Genome-Wide Human SNP array 6.0. We identified the 15q13.3 microdeletion in 7 of 647 IGE cases (**Supplementary Fig. 1** online) with different IGE syndromes (**Table 1**). All but one of these showed segmental breakpoints BP4 and BP5, as observed in the 15q13.3 microdeletion syndrome[4]. The other showed a 3.8-Mb deletion defined by BP3 and BP5. The 15q13.3 deletion was not detected in any of 1,202 controls examined.

We next examined the frequency of 15q13.3 microdeletions in a second independent sample of 576 IGE cases from Switzerland ($n = 205$), North America ($n = 133$) and Northern Europe ($n = 238$) as well as 2,497 controls from North America of predominantly European ancestry genotyped with various methods (**Supplementary Methods** online). In this sample, we found 15q13.3 microdeletions in 5 of 576 cases and 0 of 2,497 controls. Altogether, we identified 15q13.3 deletions in 12 of 1,223 IGE cases and in 0 of 3,699 controls ($P = 5.32 \times 10^{-8}$, Fisher's exact test). We used custom array CGH (10 of 12 cases; **Fig. 1**) or SNP and CNV arrays (2 of 12 cases) to verify the deletions in all IGE probands and their available first-degree family members. We identified duplications involving *CHRNA7* in 12 of 1,223 cases and 23 of 3,699 controls ($P = 0.27$, Pearson's χ^2 test; **Supplementary Methods** and **Supplementary Fig. 2** online). Thus, our results suggest that the 15q13.3 deletion only, and not the reciprocal duplication, represents a major risk factor for IGE.

In our study, parental DNA was available for 5 of 12 probands with the deletion (**Supplementary Fig. 3** online). In one individual (L1371), the deletion was apparently de novo, as we found that both clinically unaffected parents did not carry the deletion using custom array CGH. For the other four individuals, we observed parental transmission from one father and three mothers. One mother (E562M) suffered from panic disorder, a phenotype previously associated with 15q13.3 deletions[6]. The other three transmitting parents were apparently clinically unaffected, although we cannot exclude that subclinical manifestations such as age-dependent paroxysmal EEG discharges or undetected mild and remitting IGE phenotypes might have been missed. In one family (E562), both siblings carried the deletion and were affected by IGE. In family 254, the proband's brother (254B) also carried the deletion and was affected by IGE. In the family of IGE proband 1674, the 15q13.3 microdeletion was present in a brother (2376) with severe intellectual disability but without a history of seizures.

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Table 1 Phenotypic features of individuals with 15q13.3 microdeletions

Individual	Descent	Diagnosis	Seizure types	Age of onset	EEG	Cognition
EPICURE sample						
Ao67	German	JME	Myoclonus	16 y	GSW	Normal
			GTCS	19 y		
1674	German	CAE	Absence	5 y	GSW	ID
254A	German	JAE	Absence	9 y	GSW	Normal
			GTCS	13 y		
60A	German	JAE	Absence	12 y	GSW	Normal
			GTCS	14 y		
EZ1194	Austrian	JME	Absence	6 y	GSW	Normal
			Myoclonus	10 y		
			GTCS	20 y		
40281601	German	CAE	Absence	Uncertain	GSW	Normal
			GTCS	3 y		
D04u0213	Dutch	JME	Absence	4 y	Irreg. GSW	Normal
			Myoclonus	13 y	PPR	
			GTCS			
Mixed IGE sample						
E421	Northern African	JME	Myoclonus	<26 y	Irreg. GSW	Normal
			GTCS	26 y		
E435	French	JME	Myoclonus	12 y	GSW	Mild deficits
			GTCS	12 y		
L1371	German	CAE	Absence	4 y	Irreg. GSW	Normal
D07u0771	Dutch	JAE	Absence	14 y	GSW	Normal
E562	French	JME	Myoclonus	12 y	GSW	Mild ID
			GTCS	13 y		(IQ = 73)
Affected first-degree family members						
2376 (brother of 1674)	German		None			Severe ID
254B (sister of 254A)	German	JME	Absence	15 y	GSW	Normal
			Myoclonus	15 y		
E562M (mother of E562)	French		None			Panic disorder
E562B (brother of E562)	French	EGTCS	GTCS	Uncertain	Unknown	Normal

CAE, childhood absence epilepsy; JAE, juvenile absence epilepsy; JME, juvenile myoclonic epilepsy; EGTCS, idiopathic epilepsy with GTCS; GTCS, generalized tonic-clonic seizures; GSW, generalized spike-wave discharges (2.5–5.0 Hz); Irreg. GSW, irregular generalized spike-wave discharges; PPR, photo-paroxysmal response; ID, intellectual disability.

The phenotypes of most of the individuals reported here with the 15q13.3 microdeletion differ notably from those reported originally for the 15q13.3 microdeletion syndrome, in which individuals showed marked intellectual disability, seizures, growth retardation and dysmorphic features[3]. Although we observed severe intellectual disability in 1 of 12 and mild intellectual disability in 2 of 12 probands, 9 of 12 of the probands in our sample had characteristic features of IGE without dysmorphic features or intellectual disability (**Table 1**). Seizure types in these individuals included typical absence seizures, myoclonic seizures and primary generalized tonic-clonic seizures, all of them occurring at the typical age of onset. Electroencephalographic (EEG) records were available for all individuals and showed normal background activity with paroxysmal generalized spike-wave discharges, which represent the EEG hallmark of IGE.

Two studies have reported association of the 15q13.3 microdeletion with schizophrenia and related psychoses[4,5]. Consistent with our study, those studies observed intellectual disability in only a small fraction of individuals carrying the deletion. In the current study, none of the individuals with IGE carrying a 15q13.3 deletion had a history of a psychotic episode. Thus, our findings extend the phenotypic spectrum related to the 15q13.3 deletion to common IGE syndromes without the previous reported neuropsychiatric features. Taken together, the current studies reveal extensive variability in the phenotypic manifestation associated with the 15q13.3 deletion, ranging from apparently healthy individuals to severely affected individuals with a broad spectrum of neuropsychiatric disorders[3–6]. These findings imply that shared mechanisms are involved in the pathogenesis of a spectrum of seemingly unrelated neuropsychiatric disorders, and argue for a new framework for understanding complex genetic diseases.

The critical region affected by the BP4-BP5 deletions harbors at least seven genes (*ARHGAP11B*, *MTMR15*, *MTMR10*, *TRPM1*, *KLF13*, *OTUD7A* and *CHRNA7*) that might contribute to the seizure phenotype, including *CHRNA7* as the prime candidate gene. Compelling evidence suggests that the impairment of neuronal ion channel function may be pivotal to the pathology of IGE[10]. Cholinergic pathways have several important functions in the brain, and nicotinergic acetylcholine receptors containing the α7 subunit are widely expressed throughout the central nervous system[11]. These receptors are localized both pre- and postsynaptically and are thought to modulate excitatory and inhibitory pathways. In particular, *CHRNA7* is highly expressed in the reticular thalamus[12], indicating a role in modulating thalamocortical pathways, which are central to the generation of primarily

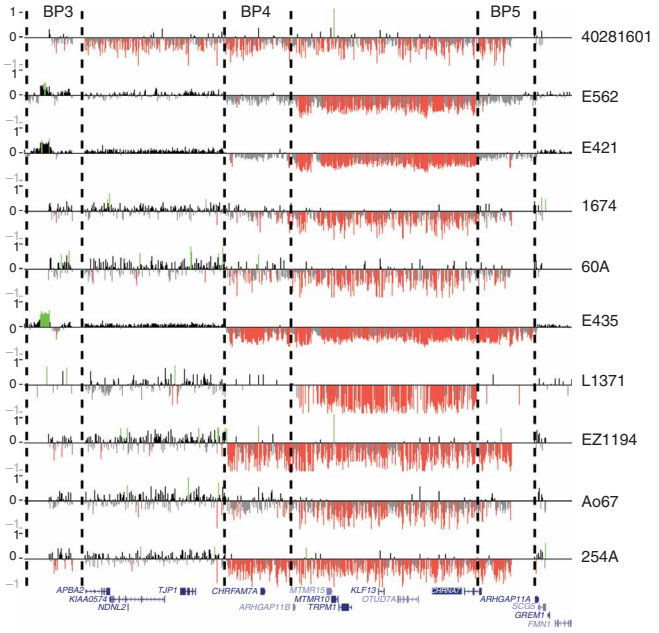

Figure 1 Confirmation of 15q13.3 microdeletions using custom array CGH. High-resolution oligonucleotide array mapping of the 15q12–q13.3 region in 10 of 12 IGE probands with 15q13.3 microdeletions. Probes with $\log_2$ ratios above or below a threshold of 1.5 s.d. are colored green (duplications) or red (deletions). Hashed lines indicate the breakpoint regions BP3–BP5.

generalized seizures seen in IGE[13]. *CHRNA2*, *CHRNA4* and *CHRNB2*, which code for the α2, α4 and β2 subunits of the nicotinergic acetylcholine receptor, respectively, have a causative role in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)[11]. Together, these lines of evidence strongly support an involvement of *CHRNA7* in epileptogenesis.

The genetic architecture of common seizure disorders is thought to show a biological continuum ranging from rare monogenic forms to common epilepsies with complex inheritance. Positional cloning of epilepsy genes has been successful in large families with monogenic inheritance, and multiple rare gene variants, including mutations in *CACNA1H* and *EFHC1*, have been found in a small proportion of individuals with IGE[10]. However, common susceptibility alleles have not been identified so far. Here we describe a structural variant that is virtually absent in the general population ($<0.02\%$)[4,5] and roughly 50 times more frequent in individuals with IGE in the present study (1%). Furthermore, the frequency of 15q13.3 deletions in IGE seems to be higher than that reported in intellectual disability or schizophrenia (**Supplementary Table 1** online). Given the strong epileptogenic effect, the 15q13.3 microdeletion represents the most prevalent major risk factor for IGE identified to date.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

T.S. and E.E.E. initiated and designed the study; I.H., H.M., S.v.S., I.S., A.A.K.-L., V.G., B.S., K.M.K., P.S.R., F.R., Y.W., H.L., F.Z., L.U., K.F., M. Feucht, F.V., G.-J.d.H., R.S.M., H.H., D. Luciano, C.R., D. Lindhout, C.E.E., U.S. and T.S. recruited and phenotyped the EPICURE sample; H.C.M., A.J.S., M.G., M. Fichera, C.B., P.G., P.T., A.M. and E.E.E. recruited and phenotyped the mixed IGE sample; A.F., M.W., M.N. and S.S. recruited and phenotyped the PopGen control sample; I.H., A.F., C.L., K.L.K., I.S., M.W., M.N., P.N. and T.S. performed the CNV analysis on SNP arrays; H.C.M., A.J.S., M. Fichera, C.B. and D. Luciano performed the qPCR screening; H.C.M., M. Fichera, C.B. and D. Luciano performed the screening using Illumina Genotyping BeadChips; H.C.M., A.J.S. and C.B. performed the confirmation using NimbleGen arrays; C.d.K., B.P.C.K. and D. Lindhout performed the confirmation using Illumina CNV BeadChips; I.H., H.C.M., A.J.S., M.G., M. Fichera, A.F., C.d.K., K.L.K., C.R., B.P.C.K., D. Lindhout, E.E.E. and T.S. coordinated the work and prepared the manuscript.

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