

15q13.3 microdeletions increase risk of idiopathic generalized epilepsy

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

Idiopathic generalized epilepsies (IGE) are common seizure disorders accounting for up to one-third of all epilepsies¹. The vast majority of IGE patients display a complex genetic aetiology², for which the underlying genetic alterations remain largely unknown. Recently, a 15q13.3 microdeletion syndrome was identified in 0.2-0.3% of individuals with mental retardation³, schizophrenia^{4,5}, autism and other neuropsychiatric features⁶. The critical region of the 1.5 Mb deletion on 15q13.3 contains at least seven genes, including the *CHRNA7* gene coding for the alpha-7 subunit of the nicotinic 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 spikes⁷ and common IGE syndromes^{8,9} have also been mapped to the 15q13-q14 region. To test whether the 15q13.3 deletion increases risk to common epilepsies, we screened for structural variants within the 15q13.3 region in two independent samples of IGE patients and ancestrally matched controls. The first study sample comprised 647 unrelated IGE patients of Western European ancestry (EPICURE sample) and 1,202 German controls (PopGen) genotyped using the Affymetrix Genome-Wide Human SNP array 6.0. The 15q13.3 microdeletion was identified in 7 out of 647 IGE patients (**Supplementary Fig. 1a, 1b** online), exhibiting different IGE syndromes (**Table 1**). All but one IGE patient showed segmental breakpoints (BP) 4 and 5, as observed in the

15q13.3 microdeletion syndrome⁴. A 3.8 Mb deletion defined by BP3 and 5 was observed in one IGE patient. The 15q13.3 deletion was not detected in any of the 1,202 controls.

We next examined the frequency of 15q13.3 microdeletions in a second independent sample of 576 IGE patients 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, 15q13.3 microdeletions were found in 5 out of 576 patients and none of 2,497 controls. Altogether 15q13.3 deletions were identified in 12 out of 1,223 IGE patients and in none of 3,699 controls ($p=5.32 \times 10^{-8}$, Fisher's Exact Test). Custom array-CGH (10 out of 12 patients; **Fig. 1**) or SNP/CNV arrays (2 out of 12 patients) were used to verify the deletions in all IGE probands and their available first-degree family members. Duplications involving *CHRNA7* were identified in 12 out of 1,223 cases and 23 out of 3,699 controls ($p=0.27$, Pearson's χ^2 test, **Supplementary Methods** online). Thus, our results suggest that only the 15q13.3 deletion represents a major risk factor for IGE.

In our study, parental DNA was available for 5 of the IGE 12 patients (**Supplementary Fig. 3** online). In one patient (L1371) the deletion was apparently *de novo*, as both clinically unaffected parents were found not to carry the deletion using custom array-CGH. Parental transmission was observed from one father and three mothers. One mother (E562M) suffered from panic disorder, a phenotype previously described with 15q13.3 deletions⁶. 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.

The phenotypes of the majority of our IGE patients differ remarkably from those reported originally for the 15q13.3 microdeletion syndrome, in which patients exhibited marked intellectual disability, seizures, growth retardation, and dysmorphic features³. While we observed severe intellectual disability in 1 out of 12 and mild intellectual disability in 2 out of 12 patients in our sample, 9 out of 12 of our patients displayed characteristic features of IGE without dysmorphic features or intellectual disability (**Table 1**). Seizure types in these patients 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 patients showing normal background activity with paroxysmal generalized spike-wave discharges, which represent the EEG hallmark of IGE.

Two studies have reported an association of the 15q13.3 microdeletion with schizophrenia and related psychoses^{4,5}. Consistent with our study, intellectual disability was observed only in a small fraction of patients carrying the deletion. None of the present IGE patients carrying a 15q13.3 deletion had a history of a psychotic episode. Thus, our present 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 an extensive variability of the phenotypic manifestation associated with the 15q13.3 deletion, ranging from apparently healthy individuals to patients severely affected with a broad spectrum of neuropsychiatric disorders³⁻⁶. These unanticipated findings imply that shared pathogenetic mechanisms are involved in the pathogenesis of a broad spectrum of seemingly unrelated neuropsychiatric disorders and argue for a new paradigm in 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* coding for the alpha-7 subunit of the nicotinic acetylcholine receptor as the prime candidate gene. Compelling evidence

suggests that the impairment of neuronal ion channel function may be pivotal to the pathology of IGE¹⁰. Cholinergic pathways have several important functions in the brain, and nicotinic acetylcholine receptors containing the alpha-7 subunit are widely expressed throughout the CNS¹¹. 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 thalamus¹², indicating a role in the modulation of thalamocortical pathways, which are central to the generation of primarily generalized seizures seen in IGE¹³. *CHRNA2*, *CHRNA4*, and *CHRNA2* which code for the alpha-2, alpha-4, and beta-2 subunits of the nicotinic acetylcholine receptor, respectively, play 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 display 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 IGE patients¹⁰. However, common susceptibility alleles have not been identified so far. Here we describe a structural variation that is virtually absent in the general population (< 0.02%)^{4,5}, but is at least 50-times more frequent in patients with IGE in the present study (1%). Furthermore, the frequency of 15q13.3 deletions in IGE appears 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.

Supplementary Information is available on the Nature Genetics website.

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Table 1 | Phenotypic features of individuals with 15q13.3 deletions

Individual	Descent	Diagnosis	Seizure Types	Age of Onset	EEG	Cognition
EPICURE Sample						
Ao67	German	JME	Myoclonus	16y	GSW	Normal
			GTCS	19y		
1674	German	CAE	Absence	5y	GSW	ID
254A	German	JAE	Absence	9y	GSW	Normal
			GTCS	13y		
60A	German	JAE	Absence	12y	GSW	Normal
			GTCS	14y		
EZ1194	Austrian	JME	Absence	6y	GSW	Normal
			Myoclonus	10y		
			GTCS	20y		
40281601	German	CAE	Absence	Uncertain	GSW	Normal
			GTCS	3y		
D04u0213	Dutch	JME	Absence	4y	Irreg. GSW	Normal
			Myoclonus	13y	PPR	
			GTCS			
Mixed IGE Sample						
E421	Northern African	JME	Myoclonus	<26y	Irreg. GSW	Normal
			GTCS	26y		

E435	French	JME	Myoclonus	12y	GSW	Mild deficits
			GTCS	12y		
L1371	German	CAE	Absence	4y	Irreg. GSW	Normal
D07u0771	Dutch	JAE	Absence	14y	GSW	Normal
E562	French	JME	Myoclonus	12y	GSW	Mild ID
			GTCS	13y		(IQ = 73)

Affected First Degree Family Members

2376 (brother of 1674)	German	-	None	-	-	Severe ID
254B (sister of 254A)	German	JME	Absence	15y	GSW	Normal
			Myoclonus	15y		
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 (2.5-5Hz); Irreg. GSW Irregular Generalized Spike-Wave discharges; PPR Photo-paroxysmal Response; ID Intellectual Disability

Figure 1 | Confirmation of 15q13.3 microdeletions using custom array-CGH.

High-resolution oligonucleotide array mapping of the 15q12-q13.3 region in 10 out of 12 IGE probands with 15q13.3 microdeletions. Probes with \log_2 ratios above or below a threshold of 1.5 standard deviations are colored green (duplications) or red (deletions). Hashed lines indicate the breakpoint regions BP3-BP5.

