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Short communication

Evaluation of multiple putative risk alleles within the 15q13.3 region for genetic generalized epilepsy

John A. Damiano[a], Saul A. Mullen[a,b], Michael S. Hildebrand[a], Susannah T. Bellows[a], Kate M. Lawrence[a], Todor Arsov[a], Leanne Dibbens[c], Heather Major[d], Hans-Henrik M. Dahl[a], Heather C. Mefford[e], Benjamin W. Darbro[d], Ingrid E. Scheffer[a,b,f], Samuel F. Berkovic[a,*]

[a] Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Heidelberg, Victoria 3084, Australia
[b] Florey Institute, Heidelberg, Victoria 3084, Australia
[c] Division of Health Sciences, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia 5000, Australia
[d] Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA
[e] Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, USA
[f] Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia

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ABSTRACT

The chromosome 15q13.3 region has been implicated in epilepsy, intellectual disability and neuropsychiatric disorders, especially schizophrenia. Deficiency of the acetylcholine receptor gene *CHRNA7* and the partial duplication, *CHRFAM7A*, may contribute to these phenotypes and we sought to comprehensively analyze these genes in genetic generalized epilepsy. We analyzed using DHPLC, Sanger sequencing and long range PCR, 174 probands with genetic generalized epilepsy with or without intellectual disability or psychosis, including 8 with the recurrent 15q13.3 microdeletion. We searched *CHRNA7* and *CHRFAM7A* for single sequence variants, small copy number variants, and the common 2-bp deletion in *CHRFAM7A*. We identified two novel and one reported missense variants. The common 2-bp deletion was not enriched in patients compared to controls. Our data suggest that missense mutations in *CHRNA7* contribute to complex inheritance in genetic generalized epilepsy in a similar fashion to the 15q13.3 microdeletion. They do not support a pathogenic role for the common 2-bp *CHRFAM7A* deletion.

1. Introduction

CHRNA7, at chromosome 15q13.3, encodes the alpha7 subunit of the neuronal nicotinic acetylcholine receptor and has long been a candidate gene for neuropsychiatric disorders. Linkage analyses and neurophysiological studies initially associated the *CHRNA7* region with schizophrenia (Freedman et al., 1997). Definitive evidence for involvement of this region in epilepsy and neuropsychiatric disorders emerged with robust demonstration of association between the 15q13.3 microdeletion (del15q13.3) and intellectual disability (ID) in 2008 (Sharp et al., 2008); then subsequently with autism spectrum disorder (ASD), schizophrenia and genetic generalized epilepsy (GGE) (Helbig et al., 2009; Miller et al., 2009; Shinawi et al., 2009; Stefansson et al., 2009). Among the associated heterogeneous phenotypes, GGE is most frequent with over 1% of patients carrying del15q13.3 (Dibbens et al., 2009; Helbig et al., 2009). In those with both intellectual disability and epilepsy consistent with GGE (ID-GGE) over 5% carry del15q13.3 (Mullen et al., 2013). Despite these remarkable carriage rates, particularly in "dual-phenotype" cases, del15q13.3 acts as a risk allele rather than a Mendelian cause of disease (Dibbens et al., 2009).

CHRNA7 and its partial duplication *CHRFAM7A*, lie within the common 15q13.3 microdeletion. As other neuronal nicotinic acetylcholine receptor subunits are known monogenic causes of epilepsy (*CHRNA4*, *CHRNA2* and *CHRNA2*), interest focused on *CHRNA7* as the likely critical gene in the 15q13.3 region (Taske et al., 2002). This was reinforced by the description of small 15q13.3 deletions encompassing *CHRNA7* and *CHRFAM7A* associated with ID (Liao et al., 2011). This is a complex region, however. The partially duplicated gene alongside *CHRNA7*, *CHRFAM7A*, comprises exons 5–10 of *CHRNA7* fused to the *FAM7A* gene. Although previously

* Corresponding author at: Epilepsy Research Centre, Level 2, Melbourne Brain Centre, 245 Burgundy St., Heidelberg, Victoria 3084, Australia.
E-mail address: s.berkovic@unimelb.edu.au (S.F. Berkovic).

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Table 1
Epilepsy syndrome diagnosis of the patient cohort screened in this study.

Epilepsy syndrome diagnosis	GGE	GGE-Plus[a]
Early onset absence epilepsy	4	26 (1 with ASD)
Childhood absence epilepsy	21	28 (4 with psychosis, 4 with ASD)
Juvenile absence epilepsy	13	6 (3 with psychosis)
Juvenile myoclonic epilepsy	20	15 (6 with psychosis, 1 with ASD)
Generalized tonic–clonic seizures alone	4	4 (2 with psychosis)
Other genetic generalized epilepsy	15	18 (2 with psychosis)
Patient total	77	97 (17 with psychosis, 6 with ASD)

[a] GGE-Plus includes cases with GGE and intellectual disability, GGE and autism spectrum disorder (ASD), and GGE and psychosis.

Table 2
Single nucleotide variants detected in *CHRNA7* and *CHRFAM7A*.

DNA	Protein	Gene	Cases	Controls	PolyPhen	Comment
c.481G>T	p.V161L	*CHRFAM7A*	1/174	0/182	Possibly damaging	This study; Not in ExAC
c.700G>A	p.G234S	*CHRNA7*	1/174	0/182	Possibly damaging	This study; Not in ExAC
c.1354G>A	p.E452K	*CHRNA7*	1/174	0/182	Benign	Reference 13; Not in ExAC

thought non-functional, more recent evidence suggests *CHRFAM7A* may affect CHRNA7 currents by regulating surface expression of the channel (de Lucas-Cerrillo et al., 2011).

A number of studies have examined sequence variation in this region. One study in 2002, seeking molecular variation responsible for a putative linkage signal at 15q13-14 in GGE, reported three rare variants, although no significant association was found (Flomen et al., 2006). Variation in *CHRNA7* and *CHRFAM7A* in schizophrenia has also been reported but its significance remains unclear (Flomen et al., 2006; Gault et al., 2003). These studies have been limited by the sequence homology between *CHRNA7* and *CHRFAM7A*. With conventional PCR it is not possible to determine, for the duplicated exons at least, the gene in which variants are located.

In addition, the contribution of a common 2-base pair (bp) deletion in *CHRFAM7A* to psychiatric disease has been suggested. This deletion is a marker of inversion of *CHRFAM7A*. An association has been claimed in some cohorts (Gault et al., 2003) but not in others (Flomen et al., 2006; Raux et al., 2002). An association with GGE has also been reported (Rozycka et al., 2013).

Here we examine sequence variation in *CHRNA7* and *CHRFAM7A* in the phenotypes most strongly associated with del15q13.3. We selected probands with either GGE alone or a "dual phenotype" including GGE and a second del15q13.3 phenotype such as ASD, schizophrenia or ID. We hypothesized that sequence variation would act either as a risk allele in a similar manner to del15q13.3 or modify the expression of a co-existing deletion of the other chromosome 15 allele. We utilized long-range PCR to reliably define in which gene variants reside. In addition, we sought to confirm the association of the common 2 bp deletion in *CHRFAM7A* with GGE.

2. Materials and methods

2.1. Subjects

We recruited 174 GGE probands: 77 had GGE alone while 97 had GGE-plus with at least one additional phenotype of ID (77), psychosis (17) or ASD (6). This included 4 previously reported GGE cases and 4 previously reported GGE-plus cases shown to carry the 15q13.3 microdeletion (Dibbens et al., 2009; Mullen et al., 2013). The breakdown in epileptic syndrome diagnosis of this cohort is shown in Table 1. We also genotyped 182 Caucasian controls for rare variants identified in this study.

Genomic DNA was extracted from venous blood by standard methods. The Human Research Ethics Committee of Austin Health, Melbourne, Australia, approved this study (Project No. H2007/02961). Informed consent was obtained from all subjects or their parents or legal guardians.

2.2. Standard PCR and denaturing high performance liquid chromatography (DHPLC)

Partially duplicated sequences (*CHRNA7* exons 5, 6, 7, 8, 9 and 10) were analyzed using the DHPLC Melt Program (http://insertion.stanford.edu/melt.html). The *CHRNA7* and *CHRFAM7A* genes were amplified using gene-specific primers (available upon request). Following PCR, heteroduplexes were formed by denaturing at 95 °C for 5 min and re-annealing at 65 °C for 30 min. For DHPLC analysis, 3 μL of each PCR product was injected and the runs were performed with a flow rate of 0.5 mL/min on a Varian Helix System (Palo Alto, CA).

2.3. Long range PCR

Since the partial duplication *CHRFAM7A* consists of exons 5 to 10 of *CHRNA7*, we used long range PCR to distinguish these genes at the 15q13.3 locus. Previously reported primers (Flomen et al., 2006) were used to specifically amplify *CHRNA7* exons 1, 2 3 and 4. Amplification reactions were cycled using Fermentas Long Range Enzyme Master Mix (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions.

2.4. Sequencing

Both standard and long range PCR products were bi-directionally sequenced using the same primers as above and a BigDye™ v3.1 Terminator Cycle Sequencing Kit (Life Technologies), according to the manufacturer's instructions. Sequencing products were resolved using a 3730xl DNA Analyzer (Life Technologies).

2.5. Multiplex-ligation dependent probe amplification (MLPA)

MLPA was performed using probes manufactured by MRC-Holland (http://www.mrc-holland.com/; Amsterdam, The Netherlands). We designed a custom probe set to target *CHRNA7* and *CHRFAM7A*. 200 ng of genomic DNA was used per MLPA reaction and products were resolved using a 3730xl DNA Analyzer (Life Technologies).

2.6. Comparative genomic hybridization (arrayCGH)

ArrayCGH was performed on Roche NimbleGen 720K whole genome tiled microarrays (Madison, WI) using standard clinical diagnostic methods. All probands had already been screened for the 1.5 kb del15q13.3 using Taqman (Dibbens et al., 2009; Mullen et al., 2013).