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***ADGRV1* is Implicated in Myoclonic Epilepsy**

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Summary

Objective: To investigate the significance of variation in *ADGRV1* (also known as *GPR98*, *MASS1* and *VLGR1*), *MEF2C*, and other genes at the 5q14.3 chromosomal locus in myoclonic epilepsy.

Methods: We studied the epilepsy phenotypes of four individuals with 5q14.3 deletion and found that all had myoclonic seizures. We then screened six contiguous genes at 5q14.3, *MEF2C*, *CETN3*, *MBLAC2*, *POLR3G*, *LYSMD3* and *ADGRV1*, in a 95-patient cohort with epilepsy and myoclonic seizures. Of these genes, point mutations in *MEF2C* cause a phenotype involving seizures and intellectual disability. A role for *ADGRV1* in epilepsy has previously been proposed, based on a recessive mutation in the Frings mouse model of audiogenic seizures, as well as a shared homologous region with another epilepsy gene, *LGII*.

Results: Six patients from the myoclonic epilepsy cohort had likely pathogenic ultra-rare *ADGRV1* variants, and statistical analysis showed that ultra-rare variants were significantly over-represented when compared to healthy population data from the Genome Aggregation Database. Of the remaining genes, no definite pathogenic variants were identified.

Significance: Our data suggest that *ADGRV1* variation contributes to epilepsy with myoclonic seizures, though the inheritance pattern may be complex in many cases. In patients with 5q14.3 deletion and epilepsy, *ADGRV1* haploinsufficiency likely contributes to seizure development. The latter is a shift from current thinking, as *MEF2C* haploinsufficiency has been considered the main cause of epilepsy in 5q14.3 deletion syndrome. In cases of 5q14.3 deletion and epilepsy, seizures likely occur due to haploinsufficiency of one or both of *ADGRV1* and *MEF2C*.

Keywords: ADGRV1, MEF2C, Myoclonic epilepsy, Chromosome 5q deletion syndrome, Frings mouse

Introduction

The 5q14.3 chromosomal locus has been a region of interest for epilepsy since linkage studies for both febrile seizures and juvenile myoclonic epilepsy indicated causative genes might lie in this vicinity.¹⁻³ However, as yet, no gene has been identified in this region to account for these phenotypes.

There is, however, a distinctive copy number variant syndrome of the 5q14.3 region. Heterozygous deletions at 5q14.3 are associated with intellectual disability, dysmorphic features, hypotonia and epilepsy (OMIM 612881). The seizure phenotype in 5q14.3 microdeletion syndrome typically involves infantile onset of febrile seizures, infantile spasms and myoclonic seizures.⁴⁻⁷ Brain structural abnormalities are common and include decreased white matter volume, periventricular heterotopia and polymicrogyria.^{8,9} A neurocutaneous syndrome involving intracranial and dermatologic vascular malformations has also been described.¹⁰⁻¹²

The underlying genetics of 5q14.3 deletion epilepsy are complex. Current literature points to *MEF2C* (OMIM 600662) haploinsufficiency playing the most important role, largely based on identification of intragenic mutations of this gene in patients with intellectual disability, stereotypies and infantile-onset epilepsy which may include infantile spasms, myoclonic, atonic, generalized tonic-clonic, focal impaired awareness, atypical absence and hemiclonic seizures.^{4,5,7,13-15} However, *MEF2C* lies outside the deleted region in at least five reported cases of 5q14.3 deletion syndrome,^{8,9,16,17} so a role for other genes at the 5q14.3 locus is likely.

A prime candidate is *ADGRV1* (OMIM 602851; previously known as *GPR98*, *MASS1*, *VLGRI*), affected in four of the five 5q14.3 deletion cases which exclude *MEF2C*. *ADGRV1* encodes adhesion G protein-coupled receptor V1, a large calcium-binding protein widely expressed in the central nervous system.^{18,19} *ADGRV1* shares some common features with an established epilepsy gene, *LGII* (OMIM 604619), associated with autosomal dominant epilepsy with auditory features.^{20,21} Both genes encode proteins which share a 7-fold repeated 44-residue motif homology domain, that has been termed an epilepsy-associated region (EAR) domain.²² In humans, *ADGRV1* mutations are associated with Usher syndrome IIC (OMIM 605472), an autosomal recessive condition involving retinitis pigmentosa and progressive hearing loss, without epilepsy.²³ A homozygous truncating mutation in the mouse ortholog, *Adgrv1*, causes audiogenic reflex seizures in the Frings mouse.²⁴ In humans, screening for *ADGRV1* mutations in 48 families with febrile seizures identified one in which a heterozygous truncating mutation was found in two affected siblings, both of whom would be classified as febrile seizures plus (FS+), as well as their unaffected father.^{25,26}

We identified three patients with chromosome 5q14.3 microdeletions; the common deletion region included 5 genes, but excluded *MEF2C*. An additional patient was identified with a complex chromosome 5 rearrangement, also resulting in a 5q14.3 microdeletion. Detailed phenotyping found that all four patients had myoclonic epilepsy. We then hypothesized that variants in genes at this locus might be present in patients with other epilepsy syndromes, and screened for variants in six genes, including *ADGRV1* and *MEF2C*, in a cohort of 95 patients with epilepsy with myoclonic seizures. Our results suggest that *ADGRV1* variation may be an important contributor to the development of genetic epilepsies, particularly those with myoclonic seizures.

Methods

5q14.3 Deletion Epilepsy Phenotyping and Molecular Analysis

We studied the epileptology of four patients with *de novo* heterozygous deletions of 5q14.3, including one with a complex chromosome 5 inversion. The boundaries of each deleted region were clarified with single nucleotide polymorphism microarray. A 100K GeneChip Affymetrix (50K Hind III; 50K XbaI) was used, and data analyzed with the GeneChip Chromosome Copy Number Analysis Tool software.

Cohort of Patients with Epilepsy with Myoclonic Seizures

A cohort of 95 patients with myoclonic seizures and epilepsy was assembled from our Epilepsy Genetics research database. Each patient underwent detailed electroclinical assessment and seizures were classified according to the International League Against Epilepsy Classification.^{27,28} The cohort was composed of 41 patients with genetic generalized epilepsy (GGE), 46 with developmental and/or epileptic encephalopathies, and eight with unclassified epilepsies.

Variant Screening in the Myoclonic Epilepsy Cohort

We used high resolution melt (HRM) analysis and/or sequencing to screen for variants in the five genes found in the common deleted region of patients 1-3 (*CETN3*, *MBLAC2*, *POLR3G*, *LYSMD3* and *ADGRV1*), as well as *MEF2C*, in the 95-patient myoclonic epilepsy cohort.

Polymerase chain reaction (PCR) and HRM nucleotide primers were determined from the Primer3 web site (<http://frodo.wi.mit.edu/primer3/>) allowing 100-200 base pairs 5' and 3' of the exonic fragment. All PCR fragments for sequencing were amplified with an annealing temperature of 60°C and the HOT star polymerase (Qiagen). HRM amplification was performed using the Light Cycler 480 and High Resolution Melting Master mix (Roche).

In order to assess whether the *ADGRV1* variants were present in controls, we screened for all identified variants in a 528-individual control group ethnically similar to our patient population (384 healthy Australian individuals, 96 individuals in the European Collection of Cell Cultures Human Random Control DNA Panel (HRC-1, Sigma) and 48 from the Coriell Cell Repository Human Variation Caucasian panel). Three multiplex MALDI_TOF sequenom assay was designed to assay all *ADGRV1* variants using the MassARRAY Assay Design 3.1. The iPLEX Gold mass extension reactions were performed according to the manufacturer's conditions (Sequenom).

A multiple ligation-dependent probe amplification (MLPA) assay was also performed in order to screen for deletions or duplications in the coding regions of *MEF2C* and *ADGRV1*. MLPA reagents were obtained from MRC-Holland and a custom-made MLPA was designed using the Human MLPA Probe Design web-based software

(<http://genomics01.arcan.stonybrook.edu/mlpa/cgi-bin/mlpa.cgi>).²⁹⁻³¹ An *ADGRV1* single-colour MLPA assay was designed with the maximum number of synthetic oligonucleotide probes targeting 13 exonic fragments from the 90 coding regions. These regions encompassed the Calx- β domains (exons 23, 70), the EAR domains (exons 44, 49), the transmembrane domains (exons 83, 85, 86, 87) and other exonic fragments (exons 3, 31, 53, 74, 78) located between the Calx- β domains in the extracellular portion of *ADGRV1*. The *MEF2C* MLPA was designed to encompass all 10 exons and included two regions within the promoter.

DNA samples with heteroduplex variants were subsequently re-amplified and sequenced to identify the variation.

Evaluating Pathogenicity of Variants

Variants were evaluated for pathogenicity based on their frequency in the Genome Aggregation Database (gnomAD)³² as well as *in silico* testing that included Polyphen-2, Mutation Taster, Genomic Evolutionary Rate Profiling and Grantham scores. Variants were classified as “ultra-rare” if they were absent in gnomAD and “rare” if AF was < 0.0005. We sequenced parental DNA when available to determine if variants were inherited or *de novo*. Classification of pathogenicity was dictated by the American College of Medical Genetics and Genomics (ACMG) standards and guidelines.³³

We undertook statistical analysis using gnomAD data to investigate whether the findings of rare and ultra-rare variants might occur by chance and not have biological significance. In the gnomAD database of ~246,000 alleles with good coverage of *ADGRV1*, there are 15765 rare missense/LOF variants with AF < 0.0005 including 1794 variants present only once. We compared the proportion of rare and ultra-rare variants in our myoclonic epilepsy population to gnomAD data using Fisher’s exact test. This statistical approach requires a simplified model in which no allele carries more than one variant; however, this is still a reasonable method to estimate whether rare and ultra-rare variants are overrepresented.

The study was approved by the Human Research Ethics Committee of Austin Health, Project No. H2007/02961. Informed, written consent was obtained from all subjects, or their legal guardian.

Results

Patients with *de novo* 5q14.3 Deletions

The clinical-molecular features of the three patients with 5q14.3 microdeletions and the fourth patient with a complex chromosome 5 inversion-deletion are summarized in Table 1. Details of the copy number variants are in Table S1; the molecular data, as well as limited clinical detail, from patients 1-3 have been published previously.^{9,34} The three straightforward deletions shared a 1.34 Mb common deleted region affecting five genes: *CETN3* (OMIM 602907); *MBLAC2*; *POLR3G*; *LYSMD3*; and *ADGRV1* (Figure). The fourth patient had a small 5q14.3 deletion (2.3 Mb) and a large inversion encompassing 5q14-23.1 (13.9 Mb). Notably, *MEF2C* was not included in the overlapping region of all four cases; in patient 3, only the *MEF2C* anti-sense RNA was affected.

Briefly, all four patients had neonatal or infantile-onset epilepsy with myoclonic and generalized tonic-clonic seizures, with the latter provoked by fever in two. Exaggerated startle was noted in patients 1 and 3, with patient 3 also having prominent reflex myoclonic seizures, triggered by auditory or tactile stimulation. Atypical absence seizures occurred in patients 2 and 4. EEG showed generalized spike-wave or polyspike-wave discharges. All patients had severe to profound developmental impairment, and were classified as having myoclonic developmental and epileptic encephalopathy. With respect to vision and hearing impairment, patient 3 had conductive hearing loss and visual impairment, and patient 2 had strabismus and horizontal nystagmus.

Variant Screening for *CETN3*, *MBLAC2*, *POLR3G*, *LYSMD3*, *MEF2C* and *ADGRV1*

Complete data regarding variants in genes in the overlap region are summarized in Table S2. For *ADGRV1*, five variants (NM_032119.3:c.530C>G, NM_032119.3:c.3268A>G, NM_032119.3:c.5857A>C, NM_032119.3:c.7342G>A, NM_032119.3:c.13949C>G) were classified as ultra-rare (absent in gnomAD) and likely damaging based on *in silico* testing (Table 2). These variants were also absent in our control group, with the exception of NM_032119.3:c.7342G>A, which was found in one control. All five variants were located in the extracellular protein domain, and all but one (NM_032119.3:c.5857A>C) were in Calx- β motifs. One variant (NM_032119.3:c.530C>G) was recurrent, present in two unrelated patients; proven *de novo* in one and bi-parental testing was not possible in the other. The six patients carrying these variants had a variety of epilepsy phenotypes (Table 2) including juvenile myoclonic epilepsy (JME; 2), Lennox-Gastaut syndrome (LGS; 2), early onset absence epilepsy with later myoclonic seizures and mild intellectual disability (1), and GGE (1). All but one of the patients had a positive family history of epilepsy in at least one first or second degree relative. Two variants were inherited, one from a mother with unclassified epilepsy and the other from a father without a history of seizures. Based on ACMG criteria, four of the variants are likely pathogenic and one is of uncertain significance.

In addition to the *ADGRV1* variants absent in gnomAD, we identified eight rare (allele frequency (AF) < 0.0005 in gnomAD) variants in seven patients which were also absent in our control

group (NM_032119.3:c.2021A>G, NM_032119.3:c.2261C>T, NM_032119.3:c.5722G>A, NM_032119.3:c.8266G>A, NM_032119.3:c.9466A>G, NM_032119.3:c.13228G>A, NM_032119.3:c.13495C>T, NM_032119.3:c.13568G>C; details in Table S3). *In silico* testing predicted seven of the variants were at least possibly damaging based on Polyphen-2 and Mutation Taster scores, with the remaining variant predicted to be benign by Polyphen-2 and Mutation Taster, but having a very high Grantham score of 194 (Table S3). These patients also had a variety of epilepsy phenotypes, including JME (2), GGE (2), LGS (2), and atypical childhood epilepsy with centrotemporal spikes with negative myoclonus (ACECTS; 1). The patient with ACECTS carried two *ADGRV1* variants (NM_032119.3:c.8266G>A and NM_032119.3:c.13228G>A). These variants were classified as of uncertain significance by ACMG criteria.

Our statistical analysis showed that based on gnomAD data, we would expect to identify ~12 rare and ~1.5 ultra-rare *ADGRV1* missense/loss of function (LOF) variants in our 95-patient cohort, thus our findings of 14 rare variants, including 6 ultra-rare, suggests an overrepresentation. Using Fisher's exact test to compare proportions, the proportions of rare variants are not statistically different ($p = 0.55$); however, there are more ultra-rare variants in the epilepsy group than would be expected ($p = 2.9 \times 10^{-3}$). This estimate suggests that the probability of this proportion of ultra-rare variants occurring by chance is less than 0.003%.

For *POLR3G*, a single ultra-rare variant was found (NM006467.2:c.532G>A), not present in gnomAD, but predicted benign by *in silico* analysis. For *LYSMD3*, a single rare variant was identified (NM_198273.2:c.769T>A), present 79 times in gnomAD (AF 0.0003), predicted possibly damaging by *in silico* analysis. Neither of these variants were considered to be pathogenic. For *CETN3*, *MBLAC2* and *MEF2C*, only known minor allelic variants were identified. MLPA did not reveal any deletions or duplications in *MEF2C* or *ADGRV1*.

Discussion

The underlying genetics of the epilepsies associated with the severe 5q14.3 deletion syndrome are complicated, as evidenced by the lack of a common deletion region. This suggests that pathogenesis is due to haploinsufficiency of multiple genes or, alternatively, that disruption of

nearby regulatory elements has effects on a specific causal gene. At present, the epilepsy has been primarily attributed to haploinsufficiency of *MEF2C*, a member of the myocyte enhancer factor-2 family of transcription factors.³⁵ Patients with intragenic *MEF2C* mutations have multiple abnormalities including intellectual disability, stereotypies and infantile-onset epilepsy which may include infantile spasms, myoclonic, atonic, generalized tonic-clonic, focal impaired awareness, atypical absence and hemiclonic seizures.^{4,5,7,13-15}

The reflex seizures in our 5q14.3 deletion cohort have not previously been reported, and are reminiscent of observations in the recessive animal model, the Frings mouse, supporting a role for *ADGRV1*. Two patients had exaggerated startle or reflex myoclonic seizures, similar to the reflex seizures that characterize the Frings mouse with *Adgrv1* mutation. Reflex myoclonic seizures have also not been reported with intragenic *MEF2C* mutations, raising the possibility that *ADGRV1* disruption may be contributing to the epilepsy phenotype in patients with 5q14.3 deletion.

We identified rare or ultra-rare *ADGRV1* variants in 13/95 (14%) of a cohort of epilepsy patients with myoclonic seizures. Six of the 95 patients (6%) carried ultra-rare (not present in gnomAD) variants that were considered to be likely pathogenic, with an additional seven patients (7%) carrying rare variants of uncertain significance. These findings suggest that *ADGRV1* may play a role in up to 6% of myoclonic epilepsies. The pattern of inheritance is likely complex in the majority; however, while some cases may contribute via an oligogenic mechanism, others may cause epilepsy in an autosomal dominant manner. Most notably, the recurrent mutation (NM_032119.3:c.530C>G) occurred *de novo* in one patient, with parental testing not available in the other, was predicted to be damaging by *in silico* testing, and was not present in gnomAD. We did not identify any likely pathogenic variants in *MEF2C* or the remaining four genes in the region.

ADGRV1 is a very large gene, encoding 90 exons and spanning 600 kb of genomic sequence;³⁶ given this, we undertook statistical analysis using gnomAD data to investigate whether the findings of rare and ultra-rare variants might occur by chance and not have biological significance. This analysis allowed us to confidently state that ultra-rare *ADGRV1* variants are

overrepresented in our myoclonic epilepsy cohort ($p = 2.9 \times 10^{-3}$) supporting our hypothesis that they are likely pathogenic.

Since homozygous *ADGRV1* mutations cause Usher syndrome IIC, the lack of reported seizures in these families appears surprising at first.³⁷ However, of reported homozygous mutations associated with Usher IIC, the vast majority result in frameshift/truncation,^{23,37-39} while we have reported only heterozygous missense mutations in our epilepsy cohort. Furthermore, Ebermann et al demonstrated that variants in *PDZD7* act as modifiers in Usher IIC, and that digenic inheritance with *PDZD7* and *ADGRV1* can occur; findings which suggested Usher IIC can be considered an oligogenic syndrome rather than having straightforward Mendelian autosomal recessive inheritance.³⁸ From a pathophysiologic perspective, Usher IIC likely occurs when the complete absence or lack of function of the ADGRV1 protein leads to dysfunction of the Usher protein network, and consequent sensorineural degeneration in the retina and inner ear.⁴⁰ In our epilepsy cohort, missense mutations presumably lead to ADGRV1 protein dysfunction which results in seizures via a different pathway that has yet to be clarified.

Although the mechanism by which *ADGRV1* haploinsufficiency leads to epilepsy remains unclear, recent animal model work may provide early insight into the nature of pathogenicity. Libé-Philippot et al recently found that *Adgrv1* is required for GABAergic interneuron development in the auditory cortex.⁴¹ Widespread cortical GABAergic cell dysfunction is a potential epileptogenic mechanism in humans which could have treatment implications; however, further work is necessary to determine if this is, in fact, the case.

In summary, based on currently available data, disruption of *ADGRV1* may be an important etiologic factor in epilepsy with myoclonic seizures, as well as other genetic epilepsy phenotypes. Moreover, in 5q14.3 deletion syndrome *ADGRV1* haploinsufficiency likely contributes to the phenotype which may involve one or more of febrile seizures, myoclonic seizures, infantile spasms, generalized tonic-clonic seizures and atypical absences. Exaggerated startle is also seen in patients with 5q14.3 deletion, and may occur due to disruption of *ADGRV1*. Point mutations in *ADGRV1* may also cause a variety of epilepsy syndromes including JME, other GGE, and LGS, though the inheritance pattern is likely complex in many individuals.

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Ethical Statement: We confirm we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Key Point Box

- 5q14.3 deletion syndrome commonly involves early onset epilepsy with myoclonic seizures, often triggered by auditory/tactile stimulation.
- Epilepsy in 5q14.3 deletion syndrome occurs due to haploinsufficiency of one or both of *ADGRV1* and *MEF2C*.
- Pathogenic variants in *ADGRV1* are a likely monogenic cause of myoclonic epilepsy.
- *ADGRV1* variation may contribute to myoclonic epilepsy pathogenesis in up to 13% of cases.

Figure Legend

Figure: Deletions in the original cohort of four patients with 5q14.3 deletions. Purple denotes the gene *ADGRV1*, and orange *MEF2C*. Figure includes a screenshot from UCSC genome browser (<http://genome.ucsc.edu>), (hg 38).

References

1. Deprez L, Claes LR, Claes KG, et al. Genome-wide linkage of febrile seizures and epilepsy to the FEB4 locus at 5q14.3-q23.1 and no MASS1 mutation. Hum Genet 2006; 118: 618-25.
2. Nakayama J, Hamano K, Iwasaki N, et al. Significant evidence for linkage of febrile seizures to chromosome 5q14-q15. Hum Mol Genet 2000; 9: 87-91.

3. Kapoor A, Ratnapriya R, Kuruttukulam G, et al. A novel genetic locus for juvenile myoclonic epilepsy at chromosome 5q12-q14. *Hum Genet* 2007; 121: 655-62.
4. Le Meur N, Holder-Espinasse M, Jaillard S, et al. MEF2C haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. *J Med Genet* 2010; 47: 22-9.
5. Paciorkowski AR, Traylor RN, Rosenfeld JA, et al. MEF2C Haploinsufficiency features consistent hyperkinesia, variable epilepsy, and has a role in dorsal and ventral neuronal developmental pathways. *Neurogenetics* 2013; 14: 99-111.
6. Nowakowska BA, Obersztyn E, Szymanska K, et al. Severe mental retardation, seizures, and hypotonia due to deletions of MEF2C. *Am J Med Genet B Neuropsychiatr Genet* 2010; 153B: 1042-51.
7. Zweier M, Gregor A, Zweier C, et al. Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish MECP2 and CDKL5 expression. *Hum Mutat* 2010; 31: 722-33.
8. Engels H, Wohlleber E, Zink A, et al. A novel microdeletion syndrome involving 5q14.3-q15: clinical and molecular cytogenetic characterization of three patients. *Eur J Hum Genet* 2009; 17: 1592-9.
9. Cardoso C, Boys A, Parrini E, et al. Periventricular heterotopia, mental retardation, and epilepsy associated with 5q14.3-q15 deletion. *Neurology* 2009; 72: 784-92.
10. Carr CW, Zimmerman HH, Martin CL, et al. 5q14.3 neurocutaneous syndrome: a novel contiguous gene syndrome caused by simultaneous deletion of RASA1 and MEF2C. *Am J Med Genet A* 2011; 155A: 1640-5.
11. Al-Shehhi M, Betts D, Mc Ardle L, et al. Jugular pit associated with 5q14.3 deletion incorporating the MEF2C locus: a recurrent clinical finding. *Clin Dysmorphol* 2016; 25: 23-6.
12. Ilari R, Agosta G, Bacino C. 5q14.3 deletion neurocutaneous syndrome: Contiguous gene syndrome caused by simultaneous deletion of RASA1 and MEF2C: A progressive disease. *Am J Med Genet A* 2016; 170: 688-93.
13. Rocha H, Sampaio M, Rocha R, et al. MEF2C haploinsufficiency syndrome: Report of a new MEF2C mutation and review. *Eur J Med Genet* 2016; 59: 478-82.

14. Bienvenu T, Diebold B, Chelly J, et al. Refining the phenotype associated with MEF2C point mutations. *Neurogenetics* 2013; 14: 71-5.
15. Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet* 2013; 45: 825-30.
16. Boutry-Kryza N, Labalme A, Ville D, et al. Molecular characterization of a cohort of 73 patients with infantile spasms syndrome. *Eur J Med Genet* 2015; 58: 51-8.
17. Shimojima K, Okumura A, Mori H, et al. De novo microdeletion of 5q14.3 excluding MEF2C in a patient with infantile spasms, microcephaly, and agenesis of the corpus callosum. *Am J Med Genet A* 2012; 158A: 2272-6.
18. Ishikawa K, Nagase T, Suyama M, et al. Prediction of the coding sequences of unidentified human genes. X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Res* 1998; 5: 169-76.
19. Nikkila H, McMillan DR, Nunez BS, et al. Sequence similarities between a novel putative G protein-coupled receptor and Na⁺/Ca²⁺ exchangers define a cation binding domain. *Mol Endocrinol* 2000; 14: 1351-64.
20. Kalachikov S, Evgrafov O, Ross B, et al. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet* 2002; 30: 335-41.
21. Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, et al. Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet* 2002; 11: 1119-28.
22. Scheel H, Tomiuk S, Hofmann K. A common protein interaction domain links two recently identified epilepsy genes. *Hum Mol Genet* 2002; 11: 1757-62.
23. Weston MD, Luijendijk MW, Humphrey KD, et al. Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am J Hum Genet* 2004; 74: 357-66.
24. Skradski SL, Clark AM, Jiang H, et al. A novel gene causing a mendelian audiogenic mouse epilepsy. *Neuron* 2001; 31: 537-44.
25. Nakayama J, Fu YH, Clark AM, et al. A nonsense mutation of the MASS1 gene in a family with febrile and afebrile seizures. *Ann Neurol* 2002; 52: 654-7.

26. Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. *Brain* 1997; 120 (Pt 3): 479-90.
27. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;
28. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 2010; 51: 676-85.
29. Ganesamoorthy D, Bruno DL, Schoumans J, et al. Development of a multiplex ligation-dependent probe amplification assay for diagnosis and estimation of the frequency of spinocerebellar ataxia type 15. *Clinical chemistry* 2009; 55: 1415-8.
30. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic acids research* 2002; 30: e57.
31. Zhi J, Hatchwell E. Human MLPA Probe Design (H-MAPD): a probe design tool for both electrophoresis-based and bead-coupled human multiplex ligation-dependent probe amplification assays. *BMC genomics* 2008; 9: 407.
32. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; 536: 285-91.
33. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405-24.
34. Slater HR, Bailey DK, Ren H, et al. High-resolution identification of chromosomal abnormalities using oligonucleotide arrays containing 116,204 SNPs. *Am J Hum Genet* 2005; 77: 709-26.
35. Leifer D, Krainc D, Yu YT, et al. MEF2C, a MADS/MEF2-family transcription factor expressed in a laminar distribution in cerebral cortex. *Proc Natl Acad Sci U S A* 1993; 90: 1546-50.

36. McMillan DR, Kayes-Wandover KM, Richardson JA, et al. Very large G protein-coupled receptor-1, the largest known cell surface protein, is highly expressed in the developing central nervous system. *J Biol Chem* 2002; 277: 785-92.
37. Hilgert N, Kahrizi K, Dieltjens N, et al. A large deletion in GPR98 causes type IIC Usher syndrome in male and female members of an Iranian family. *J Med Genet* 2009; 46: 272-6.
38. Ebermann I, Phillips JB, Liebau MC, et al. PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. *J Clin Invest* 2010; 120: 1812-23.
39. Besnard T, Vache C, Baux D, et al. Non-USH2A mutations in USH2 patients. *Hum Mutat* 2012; 33: 504-10.
40. Lentz J, Keats B, Usher Syndrome Type II, in GeneReviews(R), M.P. Adam, et al., Editors. 1993: Seattle (WA).
41. Libé-Philippot B, Michel V, Boutet de Monvel J, et al. Auditory cortex interneuron development requires cadherins operating hair-cell mechanoelectrical transduction. *Proc Natl Acad Sci U S A* 2017; 114: 7765-74.
42. Winawer MR, Marini C, Grinton BE, et al. Familial clustering of seizure types within the idiopathic generalized epilepsies. *Neurology* 2005; 65: 523-8.
43. Marini C, Scheffer IE, Crossland KM, et al. Genetic architecture of idiopathic generalized epilepsy: clinical genetic analysis of 55 multiplex families. *Epilepsia* 2004; 45: 467-78.
44. Mefford HC, Yendle SC, Hsu C, et al. Rare copy number variants are an important cause of epileptic encephalopathies. *Ann Neurol* 2011; 70: 974-85.

Tables

Table 1: Clinical Features of Patients with 5q14.3 Deletions.

#/Sex/ Age/Deletion size	Sz Onset	Seizure Types; EEG	Epilepsy Course	Development	Brain MRI	Co-morbidities	Syndrome
1/Male/ 12 y/7.0 Mb	Birth	M, FS; GSW, PSW	Sz controlled on VPA.	Profound ID. No regression.	Normal.	Dysmorphic features, intermittent tremor, severe spasticity	Myoclonic developmental and epileptic encephalopathy
2/Male/ 7 y/8.8 Mb	Birth	M, AtA; GSW, PSW	Sz controlled on VPA and CLN.	Severe ID. Regression following surgical procedures.	Normal.	Dysmorphic features, strabismus, horizontal nystagmus, cleft palate, atrial and ventricular septal defects, patent ductus arteriosus,	Myoclonic developmental and epileptic encephalopathy

						inguinal herniae, cryptorchidism.	
3/Male/ 5 y/6.3 Mb	10 m	FS, GTC, M; Independent and bilateral bursts of posterior SW.	Sz controlled on VPA.	Severe DD.	Reduced occipito- parietal white matter volume, splenium hypoplasia, and band-like periventricular heterotopia lining the occipital horns.	Conductive hearing loss, visual impairment, trigonocephaly, right postaxial polydactyly of toes, truncal hypotonia.	Myoclonic developmental and epileptic encephalopathy
4/Male/ 9 y/2.3 Mb*	4 m	M, AtA, GTC; Independent and bilateral centropari- etal ShW	Sz controlled on VPA, weaned at 7 y. Now sz- free off medication.	Severe ID. Multiple regressions with increases in seizure frequency.	Mild asymmetry of lateral ventricles	Hypersalivation, discoid eczema, coarctation of aorta, bicuspid aortic valve, perimembranous ventricular septal defect, abnormal hands with adducted (clapsed) thumbs, truncal hypotonia.	Myoclonic developmental and epileptic encephalopathy

Notes: Patient 3 has previously been reported.⁹ * Patient 4 had a complex chromosomal rearrangement as well (Table S1).

Abbreviations: AtA (Atypical absence); CLN (Clonazepam); DD (Developmental delay); EEG (Electroencephalogram); FS (Febrile seizures); GSW (Generalized spike-wave); GTC (Generalized tonic-clonic); ID (Intellectual disability); M (Myoclonic); MRI (Magnetic

resonance imaging); PSW (Polyspike-wave); ShW (Sharp-slow wave); SW (Spike-wave); VPA (Valproate)

Table 2: Clinical Features of Patients with Ultra-rare *ADGRVI* Variants (absent in gnomAD).

#	Variant	Inherited?	Clinical Phenotype	Seizure Types	Family History?
15	c.530C>G; p.T177S	<i>De novo</i>	LGS	GTC, FIA, T, A	- FS in 2 maternal aunts and 6 maternal 1 st cousins. - Unclassified “convulsions” in paternal GF, maternal 1 st cousin and maternal 2 nd cousin.
78	c.530C>G; p.T177S	Unknown	JME	M, GTC	- Mother: JME with GTC and myoclonic seizures. - Sister: Unclassified epilepsy with FS,

					myoclonic, GTC and absence/FIA. Had daughter also with unclassified epilepsy.
9	c.3268A>G; p.I1090V	Unknown	EOAE + ID	A, M	- Mother: Unclassified epilepsy. - Brother and sister: Unclassified epilepsy + mild ID.
83	c.5857A>C; p.S1953R	Mother	GGE + ID	M, GTC, AtA	- Mother: Unclassified epilepsy.
75	c.7342G>A; p.A2448T	Unknown	LGS	FS, GTC, MA, At, AtA, M	- Paternal first cousin: Unclassified epilepsy with ID.
37	c.13949C>G; p.S4650C	Father	JME	GTC, M, A	- Sister: JAE

Notes: Where inheritance is listed as “unknown” parental DNA was not available for testing. Patient 78 was previously published (JME-XV).^{42,43} For #75, the c.7342G>A (p.Ala2448Thr) variant is absent in gnomAD but was found in one of the study controls; this patient also has a *de novo* 7q11-21 deletion, previously published.⁴⁴

Abbreviations: A (Absence); At (Atonic); AtA (Atypical absence); EOAE (Early onset absence epilepsy); FIA (Focal impaired awareness); FS (Febrile seizures); GF (Grandfather); GTC (Generalized tonic-clonic); ID (Intellectual disability); GGE (Genetic generalized epilepsy); JAE (Juvenile absence epilepsy); JME (Juvenile myoclonic epilepsy); LGS (Lennox-Gastaut Syndrome); M (Myoclonic); MA (Myoclonic absence); T (Tonic)

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