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Diverse genetic causes of polymicrogyria with epilepsy

Epilepsy Phenome/Genome Project,

Epi4K Consortium

Abstract

Objective: We sought to identify novel genes and to establish the contribution of known genes in a large cohort of patients with nonsyndromic sporadic polymicrogyria and epilepsy.

Methods: We enrolled participants with polymicrogyria and their parents through the Epilepsy Phenome/Genome Project. We performed phenotyping and whole exome sequencing (WES), trio analysis, and gene-level collapsing analysis to identify de novo or inherited variants, including germline or mosaic (postzygotic) single nucleotide variants, small insertion-deletion (indel) variants, and copy number variants present in leukocyte-derived DNA.

Results: Across the cohort of 86 individuals with polymicrogyria and epilepsy, we identified seven with pathogenic or likely pathogenic variants in *PIK3R2*, including four germline and three mosaic variants. *PIK3R2* was the only gene harboring more than expected de novo variants across the entire cohort, and likewise the only gene that passed the genome-wide threshold of significance in the gene-level rare variant collapsing analysis. Consistent with previous reports, the *PIK3R2* phenotype consisted of bilateral polymicrogyria concentrated in the perisylvian region with macrocephaly. Beyond *PIK3R2*, we also identified one case each with likely causal de novo variants in *CCND2* and *DYNC1H1* and biallelic variants in *WDR62*, all genes previously associated with polymicrogyria. Candidate genetic explanations in this cohort included

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AUTHOR CONTRIBUTIONS

A.S.A., S.F.B., P.Co., N.D., D.D., E.E.E., M.P.E., C.F., D.B.G., R.G., E.L.H., M.R.J., R.Kuz., D.H.L., A.G.M., H.C.M., T.J.O., R.O., A.P., S.Petrou, S.Petrov., I.E.S., and E.H.S. designed arms of study as part of the Epi4K Steering Committee. Genetic analysis was performed by A.S.A., V.A., S.F.B., D.B.G., E.L.H., H.C.M., S.Petrov., A.P., and E.K.R. The manuscript was written by E.L.H. and A.P. Patient collection, phenotyping of study participants, and interpretation of phenotypic data were performed by S.F.B., D.H.L., H.C.M., A.P., E.H.S., B.A.-K ., D.A., E.A., F.A., J.Ba., A.B., G.C., D.C., P.Co., P.Cr., O.D., N.F., D.F., E.G., S.G., K.H., S.He., S.J., H.K., R.Kn., E.K., P.V.M., R.O., J.M.Pao., J.M.Par., I.S., R.A.S., J.J.S., S.S., R.K.S., M.S., M.C.S., J.Si., E.P.G.V., G.K.V.A., P.W.-W., M.R.W., J.Bl., M.F., T.G., J.H., S.Ha., K.P., J.Su., L.L.T., A.V., J.W., R.Kup., R.Kuz., S.M., E.N., and L.S. The collaborative activities of Epi4K Consortia were overseen by S.F.B., D.B.G., and D.H.L.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

CONFLICT OF INTEREST

D.B.G. is a founder of and holds equity in Q State Biosciences and Praxis Therapeutics; holds equity in Apostle, and serves as a consultant to AstraZeneca and Gilead. S.Petrov. has equity in and is employed by AstraZeneca. O.D. receives grant support from NINDS, NIMH, MURI, CDC, and NSF. He has equity and/or compensation from the following companies: Privateer Holdings, Tilray, Receptor Life Sciences, Qstate Biosciences, Tevard, Empatica, Egg Rock/Papa & Barkley, Rettco, SilverSpike, and California Cannabis Enterprises. He has received consulting fees from GW Pharma, Cavion, Zogenix, and Eisai. R.A.S. receives research funding from PCORI, NIH, the Pediatric Epilepsy Research Foundation, and the University of Michigan. She serves as a consultant for the Epilepsy Study Consortium, receives royalties from UpToDate for authorship of topics related to neonatal seizures, and serves as an Associate Editor for *Neurology*. L.S. is funded by the Health Research Council of New Zealand and Cure Kids New Zealand. She is a consultant for the Epilepsy Consortium and has received travel grants from Seqirus and Nutricia. She has received research grants and consulting fees from Zynerba and consulting fees from Eisai. None of the other authors has any conflict of interest to disclose.

single nucleotide de novo variants in other epilepsy-associated and neurodevelopmental disease-associated genes (*SCN2A* in two individuals, *GRIA3*, *CACNA1C*) and a 597- kb deletion at 15q25, a neurodevelopmental disease susceptibility locus.

Significance: This study confirms germline and postzygotically acquired de novo variants in *PIK3R2* as an important cause of bilateral perisylvian polymicrogyria, notably with macrocephaly. In total, trio-based WES identified a genetic diagnosis in 12% and a candidate diagnosis in 6% of our polymicrogyria cohort. Our results suggest possible roles for *SCN2A*, *GRIA3*, *CACNA1C*, and 15q25 deletion in polymicrogyria, each already associated with epilepsy or other neurodevelopmental conditions without brain malformations. The role of these genes in polymicrogyria will be further understood as more patients with polymicrogyria undergo genetic evaluation.

Keywords

de novo variant; epilepsy; exome sequencing; polymicrogyria; trio

1 | INTRODUCTION

Polymicrogyria is a developmental brain malformation characterized by the radiographic appearance of excessive and small folds of the cerebral cortex and pathological evidence of abnormal neuronal organization. Patients present with a range of clinical findings, including epilepsy, focal weakness, and oromotor apraxia. Polymicrogyria may affect the entire cerebral cortex or distinct brain regions, either bilaterally or unilaterally.[1,2] Although polymicrogyria can be due to nongenetic factors, including in utero ischemia and congenital cytomegaloviral infections,[3–5] some cases have been suspected to have a genetic basis, as polymicrogyria has been observed in families[6–8] and because it often presents with bilateral, regionally specified abnormalities, suggesting the involvement of genes expressed in specific distributions. Among the earliest polymicrogyria genes identified were *GPR56* in individuals with bilateral frontoparietal polymicrogyria,[9] *TUBA1A* with perisylvian polymicrogyria,[10] and *LAMC3* with occipital polymicrogyria.[11] The early identification of polymicrogyria as part of genetic syndromes associated with copy number variations (CNVs), such as the 22q11 deletion syndrome,[12] was followed by studies identifying a number of recurrent CNVs associated with polymicrogyria.[13] More recently, polymicrogyria has been noted in patients with overgrowth syndromes that include megalencephaly and dysmorphic features.[14,15]

The genes were discovered through the study of rare large multiplex families, small cohorts of patients with polymicrogyria in the setting of multisystem syndromes, case reports of whole exome sequencing (WES) findings, and targeted sequencing of candidate genes in polymicrogyria cohorts. To our knowledge, there have been no prior unbiased genome-wide sequencing studies of large polymicrogyria cohorts to evaluate the contribution of known and novel genes.

In this study, we aimed to perform trio-based WES in a multicenter, international cohort of patients with polymicrogyria and epilepsy recruited and extensively phenotyped through

the Epilepsy Phenome/Genome Project (EPGP)[2,16] and sequenced through the Epi4K Consortium.[17]

2 | MATERIALS AND METHODS

Patients with epilepsy and polymicrogyria on brain magnetic resonance imaging (MRI) were enrolled through the EPGP, and blood samples were collected from probands and their parents[16]; the research presented was approved by the institutional review boards at all 26 participating enrollment sites. All MRIs were reviewed by the EPGP MRI Core for eligibility. The cohort has been previously described[2]; additional phenotypic data were reviewed for this report. Blood samples were collected from patients and parents; DNA was obtained from the Coriell Institute DNA Bank (Table S1). Written informed consent was provided by patients, or their parents or legal guardians if the patient was underage.

We performed WES on 86 trios with either SeqCap EZ Exome v3 or SureSelect Human All Exon–65MB exome capture kits at the Institute for Genomic Medicine (IGM), Columbia University. De novo variants were called within trios using GATK multisample calling in the protein-coding regions (CCDS, release 14, GRCh37.p13) as described previously.[18,19] De novo variants were either Sanger validated in the probands and confirmed to be absent in parents in primary DNA, or predicted with high confidence using a validated machine-learning model using variant-level, individual-level, and genomic features to identify de novo variants (Table S2).[19] Controls (n = 2711) used for single nucleotide variant (SNV) and indel calling and gene-level collapsing included individuals sequenced at the IGM for other studies.

In addition to de novo variants, we compiled rare inherited, hemizygous, homozygous, and compound heterozygous variants. We first listed all rare (minor allele frequency [MAF] < 1% among internal controls or any population in Exome Variant Server, Exome Aggregate Consortium [release 0.3], and gnomAD browser [v2.1.1]), putatively protein-a ltering (missense [PolyPhen-2 probably/possibly damaging], nonsense, or indels) variants residing in protein-coding regions. Genotypes were excluded if they had a quality score less than 30 or genotype quality score less than 20 in the proband. We required a minimum of 10-fold coverage at a variant site to call a homozygous genotype, and the same coverage in addition to at least five reads supporting the alternate allele to call a heterozygous variant. Inherited heterozygous variants were required to have a variant allele frequency (VAF) between 40% and 60%. For rare inherited variants, we included only those absent in the aforementioned control databases; newly hemizygous (present in proband, absent in mother), homozygous, and compound heterozygous variants were required to have a homozygote frequency of zero in all control databases listed above. Candidate variants are listed in Tables S3 and S4.

We evaluated for de novo or inherited CNVs using the CoNIFER pipeline as previously described.[20,21]

Variants were assessed for pathogenicity using the principles outlined by the American College of Medical Genetics.[22]

To assess whether inherited rare variation either augments the risk conferred by de novo alleles or confers risk independently, we performed a gene-level collapsing analysis comparing, for each protein-coding gene, the fraction of cases versus the fraction of controls with a rare, functional variant. We performed three separate case–control analyses comparing (1) ultrarare (absent from control databases), functional (missense and loss-of-function) variants; (2) ultrarare, loss-of-function variants only; and (3) rare (MAF < .1%) biallelic variants. We used a previously published method with a modification to allow for mosaic variants in the ultrarare comparisons.[19]

3 | RESULTS

Among 86 individuals (39 male, 47 female) with polymicrogyria and epilepsy, 10 probands (12%) had a pathogenic or likely pathogenic de novo variant identified by individual case analysis (Table 1). Seven patients harbored heterozygous de novo variants in *PIK3R2*: five with the recurrent pathogenic PIK3R2 variant Gly373Arg, one with PIK3R2 Lys376Glu (pathogenic), and one with Asp557Tyr (likely pathogenic). Three of the seven had VAFs between 13% and 16%, which deviates from the 50% expected for a heterozygous variant (binomial probability distribution), a finding consistent with mosaicism due to the mutational event occurring during early postzygotic development.

All seven *PIK3R2* patients had bilateral polymicrogyria, most prominent in the perisylvian region (Figure 1, Table 1). Six patients presented with focal epilepsy, with onset between 15 months and 9 years, and the other one presented with infantile spasms at 3 months of age. There was a wide developmental range among them; some had normal milestones or mild delays, whereas others were severely impaired. Given the association between this gene and overgrowth syndromes involving brain malformations (hemimegalencephaly, megalencephaly–capillary malformation syndrome, and megalencephaly–polydactyly–polymicrogyria–hydrocephalus),[15,23] we specifically evaluated for other features of these syndromes. All patients displayed macrocephaly with occipitofrontal circumference measuring from +3.1 to +7.1 standard deviation (SD) greater than the mean for age for those with available measurements (Table 1). None had capillary malformations of the skin, digital abnormalities, or other syndromic features. Interestingly, there was no correlation of VAF with the extent of brain malformation or other features, meaning that the patients with mosaic *PIK3R2* pathogenic variants were not more mildly affected.

We observed de novo variants in one case each of *CCND2*, associated with bilateral perisylvian polymicrogyria (BPP) and enlarged ventricles on MRI,[24] and *DYNC1H1*, associated with BPP.[25] We identified a single case with biallelic variants in the gene *WDR62*, previously associated with microcephaly and a range of brain malformations[26]; our case also presented with microcephaly. The phenotypic features of these three individuals are consistent with the reported literature for the corresponding genes (Table 1).

Among 86 trios, we identified 99 de novo variants (average = 1.15 per trio; Table S2). Three genes were found to harbor de novo variants in multiple probands: *PIK3R2* ($n = 7$, described above), *SCN2A* ($n = 2$), and *AASDH* ($n = 2$, variants predicted to be benign).

Only *PIK3R2* had more than expected to occur by chance given the size of the cohort, gene size, and mutability, and correcting for the ~18K protein- coding genes evaluated in this study (FitDNM,[27] $p = 3.6 \times 10^{-24}$). Beyond the enrichment of rare variants in *PIK3R2* in polymicrogyria cases in the ultrarare functional collapsing analysis (Fisher exact test $p = 9 \times 10^{-10}$), no other gene was significantly enriched in cases versus controls across any of the models (Figure 2, Table S5).

To estimate aggregate genetic signal attributed to de novo variation, we performed "hot-zone" analysis as described previously,[18,19] to compare the fraction of cases versus controls harboring a highly likely damaging de novo variant in genes intolerant to functional variation, as estimated using the Residual Variation Intolerance Score method.[28] We observed that 26% of polymicrogyria probands had a highly likely damaging de novo variant, compared to 9.6% of previously published control trios ($p = .04$).[29] The increased de novo variant burden among polymicrogyria cases is largely attributed to the known or likely pathogenic de novo variants above, as the percentage dropped to 15% ($p = .47$) following the removal of the 10 genetically explained individuals.

Despite the majority of aggregate de novo variant burden being attributed to variants in *PIK3R2*, *CCND2*, and *DYNC1H1,* a small number of additional likely de novo candidates were also identified (Table S2). We identified two de novo likely pathogenic missense variants in *SCN2A*, both of whom had neonatal epilepsy presenting at 1 month of age; one had focal seizures, and the other had tonic seizures and burst suppression on electroencephalogram, consistent with Ohtahara syndrome. We identified a de novo variant in *CACNA1C*, a gene reported in the context of Timothy syndrome, features of which were not reported in our case. Similarly, we identified a de novo variant in *GRIA3*, a gene associated with intellectual disability, in a normocephalic child with BPP, normal early developmental milestones, and school difficulty recognized only at age 6 years. The *CACNA1C* and *GRIA3* variants are predicted to be likely pathogenic in their known disease contexts, but because these genes are not associated with polymicrogyria and our cases did not display the recognized gene- associated phenotypes, we classified the variants as variants of uncertain significance.

In addition to SNVs, we identified a rare de novo 597-kb deletion on chromosome 15q25.2 encompassing nine genes in one individual with polymicrogyria, focal seizures with onset at 18 years, and normal development (Figure 3). This locus has been reported as a rare susceptibility allele for neurodevelopmental disorders,[30,31] although no reported individuals with a 15q25.2 deletion have polymicrogyria. We classified the 15q25.2 deletion as a variant of unknown significance (VUS).

Rare inherited, hemizygous, homozygous, and compound heterozygous variants identified across the cohort are presented in Tables S3 and S4.

4 | DISCUSSION

We report the genetic findings of a large cohort of patients with polymicrogyria and epilepsy analyzed with a trio-based, genome-wide approach and analyze the phenotypes

of the patients with notable genetic findings. Ten of the 86 individuals studied had variants in the known polymicrogyria-related genes *PIK3R2*, *CCND2*, *DYNC1H1*, and *WDR62*, comprising 12% of our cohort. In cohort-wide analyses, including gene-level assessments of genome-wide enrichment of de novo variants and overall rare variant enrichment, only *PIK3R2*, a gene previously implicated in polymicrogyria,[15,32–34] was significantly associated with polymicrogyria. The seven individuals with rare germline or early mosaic de novo variants in *PIK3R2* had bilateral, mostly perisylvian, polymicrogyria and macrocephaly, consistent with the phenotype associated with genes in the PI3K- AKT-mTOR pathway.[15,32– 34] Note that the onset and epilepsy phenotype varied, with one patient presenting with infantile spasms at 3 months and the other six with focal epilepsy between 15 months and 9 years. Earlier seizure onset did not correlate with the degree of developmental delay or intellectual disability.

Although the genes *CCND2, DYNC1H1*, and *WDR62* did not reach statistical significance for an association with polymicrogyria in our cohort-wide analysis, we considered the variants in these three genes pathogenic or likely pathogenic given in silico predictions, phenotype, and the reported association with polymicrogyria in the literature. Specifically, the presence of macrocephaly in the patient with the *CCND2* variant and microcephaly in the patient with the *WDR62* biallelic variants are consistent with these genes' described phenotypes.

An additional five individuals with polymicrogyria harbored variants in genes or loci that are currently associated with epilepsy or other neurodevelopmental conditions but not definitively with brain malformations. Two had de novo likely pathogenic variants in *SCN2A*, the gene encoding the voltage-gated sodium channel alpha 2 subunit, which is associated with epilepsy, intellectual disability, and autism spectrum disorder.[35,36] The two variants in *SCN2A* were predicted to be likely pathogenic, consistent with the patients' neonatal epilepsy presentations, as *SCN2A* is a known cause of Ohtahara syndrome. Although *SCN2A's* connection to polymicrogyria is emerging,[35,37] we could not conclude definitively that the *SCN2A* variants contributed to the brain malformation in these patients. One case had a de novo variant in *CACNA1C*, and another case had a de novo variant in *GRIA3*, each predicted to be likely pathogenic. As these genes are associated with clinical phenotypes unrelated to polymicrogyria, we considered the variants to be VUSs. Our findings add to emerging reports implicating ion channel genes in the pathogenesis of polymicrogyria (e.g., *SCN3A* and *GRIN2D*[38–41]). Finally, we identified one patient with polymicrogyria and epilepsy with a deletion at 15q25.2. These genes and this CNV locus do not have a definitive prior association in the literature with polymicrogyria, so we consider their variants, even when compelling in other phenotypic contexts, as VUSs with respect to polymicrogyria.

Although polymicrogyria can occur in isolation or in conjunction with other features, we note that our study required that individuals have polymicrogyria and epilepsy as well as both parents available for DNA samples. It may be informative to compare our results with those in cohorts with different ascertainment strategies, for example, those studies of polymicrogyria not requiring that patients have epilepsy or including individuals with more syndromic presentations.

In summary, exome sequencing in a large cohort with polymicrogyria and epilepsy provided a likely causative explanation for 12% of the patients and a possible genetic cause for an additional 6%. Our findings support a role for genetic testing in the clinical setting for patients with polymicrogyria, particularly those with epilepsy. Specifically, polymicrogyria with macrocephaly suggests that a germline or mosaic variant in *PIK3R2*, or a related PI3K-AKT-mTOR pathway gene, may be involved. As treatment trials emerge for neurodevelopmental disorders related to this pathway, a genetic diagnosis may provide the opportunity for patients with polymicrogyria to participate in clinical trials. Other candidate polymicrogyria genes will require sequencing of additional cohorts to validate their role in polymicrogyria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

Appendix

APPENDIX 1

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Epilepsy Phenome/Genome Project

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Key Points

- Polymicrogyria is a developmental brain malformation associated with epilepsy with heterogeneous etiologies, including genetic causes
- De novo variants in *PIK3R2* represent an important cause of polymicrogyria, particularly perisylvian polymicrogyria with megalencephaly
- Additional polymicrogyria-associated variants identified in our cohort include *CCND2*, *DYNC1H1*, and *WDR62*
- Other genes and copy number variants, including *SCN2A*, traditionally associated with epilepsy may also be associated with polymicrogyria

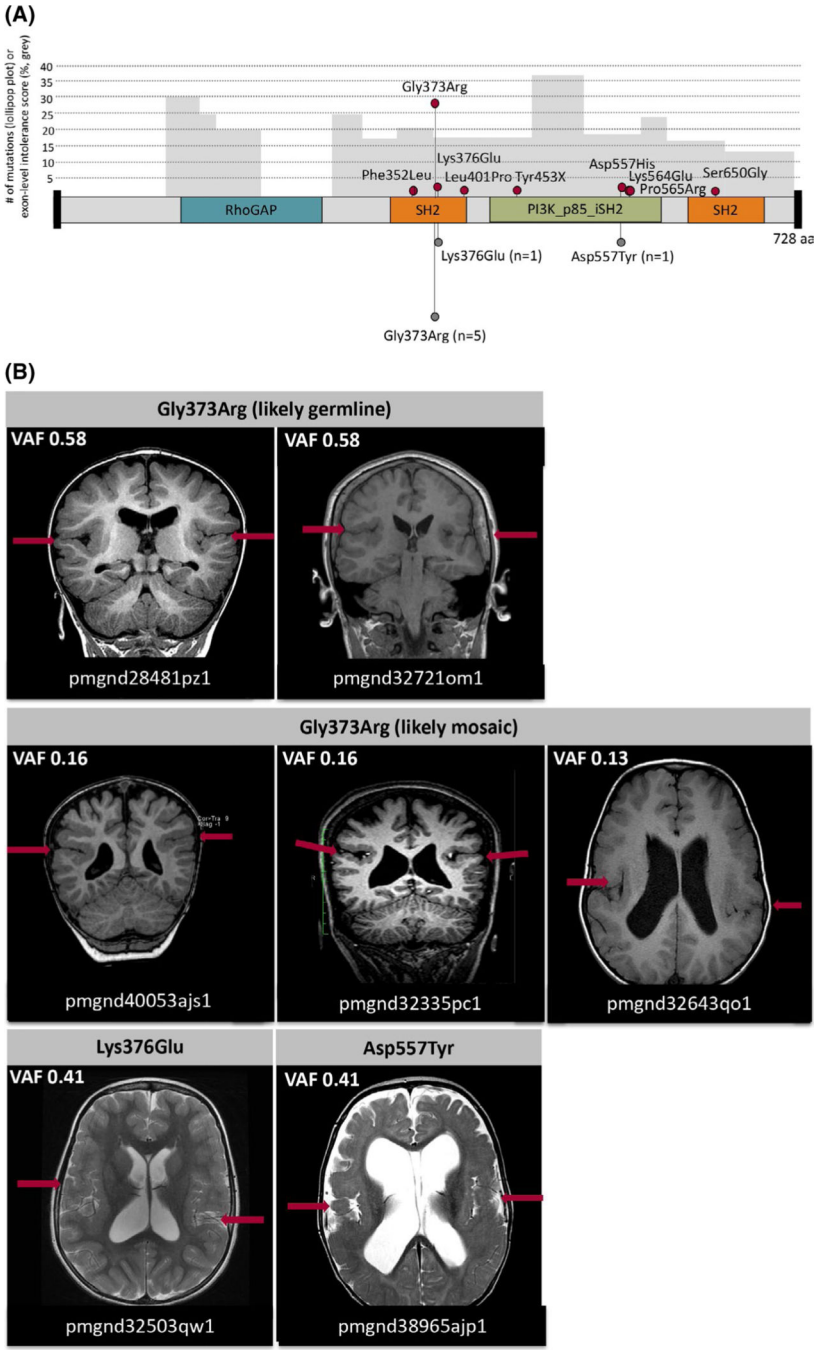

FIGURE 1.

(A) Location of previously reported brain malformation variants in *PIK3R2* are shown[15,32–34] in maroon above the protein diagram with the number of times the variant has been reported. Below the protein diagram are the variants identified in this study. Gray shading indicates the exon level intolerance score (LIMBR[42]). Higher numbers indicate more tolerance to functional variation. Both previously reported and new cases are enriched in regions of lower tolerance. (B) Magnetic resonance images from *PIK3R2* cases. VAF, variant allele frequency

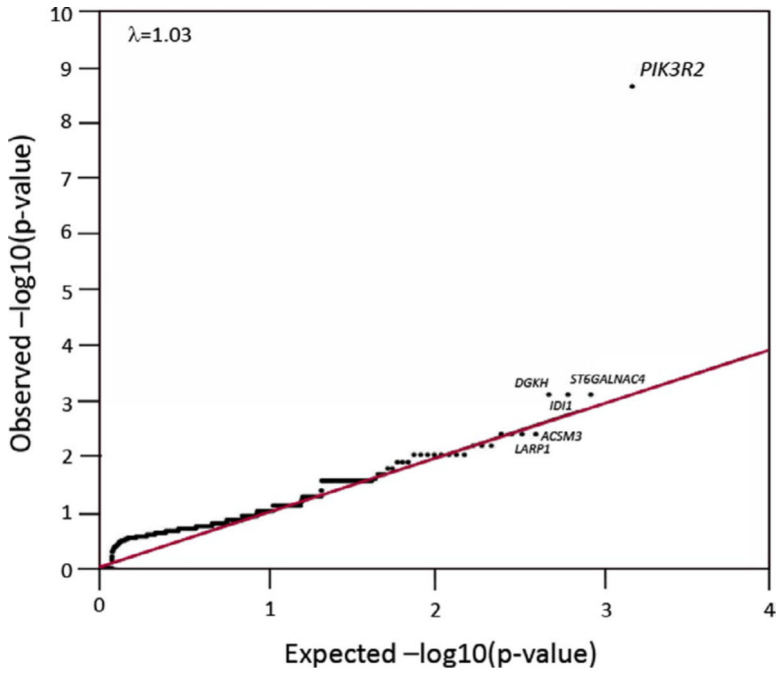

FIGURE 2.

Quantile-quantile plot for gene-level association tests interrogating ultrarare functional variants. Black dots represent transformed *p* values against the expected transformed *p* values for genes with qualifying variants. The red line indicates the expectation under the null model of no effect on risk

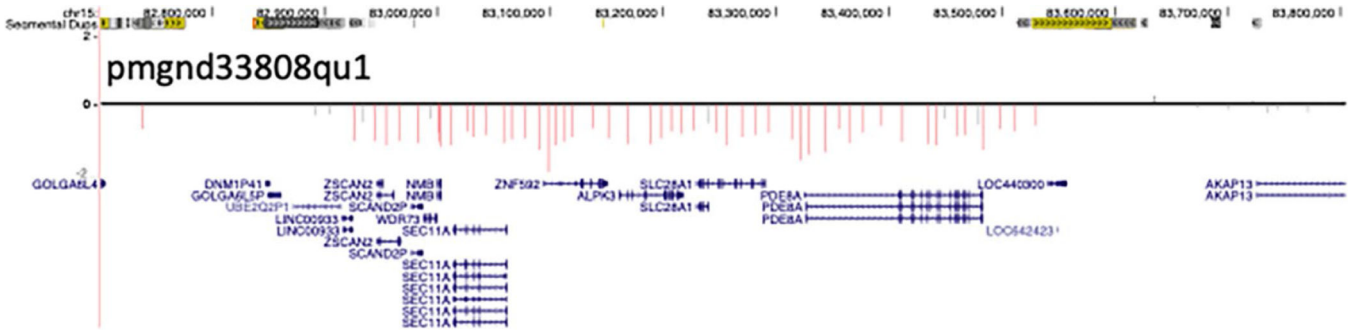

FIGURE 3.

De novo 597-k b deletion at 15q25 in pmgnd33808qu1

TABLE 1

PMG and related phenotypes for patients with pathogenic or likely pathogenic de novo variants

Patient	Genetic variant (ACMG classification) (VAF)	Sex	MRI findings	Epilepsy (onset)	Other findings
pmgnd28481pzl	PIK3R2 Gly373Arg (P) (58%)	F	BPP (L > R)	Focal epilepsy (9 years)	Macrocephaly, OFC +3.4 SD (7 week); global delay (nonverbal)
pmgnd32643qol	PIK3R2 Gly373Arg (P) (13%)	M	BPP ventriculomegaly	Focal epilepsy (7 years)	Macrocephaly, OFC +4.3 SD (7 years); speech delay, resolved
pmgnd32335pcl	PIK3R2 Gly373Arg (P) (16%)	F	BGP	Focal epilepsy (7 years)	Macrocrania
pmgnd32721oml	PIK3R2 Gly373Arg (P) (58%)	F	BPP, posterior predominant	Focal epilepsy (3 years)	Macrocephaly, OFC +6.1 SD (2.5 years); normal early milestones
pmgnd40053ajs1	PIK3R2 Gly373Arg (P) (16%)	M	BPP ventriculomegaly	Focal epilepsy (15 months)	Macrocephaly, OFC +3.2 SD (birth), +7.1 SD (8 years); mild developmental delay
pmgnd32503qwl	PIK3R2 Lys376Glu (P) (41%)	F	BPP ventriculomegaly	Focal epilepsy (6 years)	Macrocephaly, shunted for hydrocephalus; language delay
pmgnd38965ajpl	PIK3R2 Asp557Tyr (LP) (41%)	F	BPP ventriculomegaly	Infantile spasms, focal epilepsy (3 months)	Macrocephaly, OFC +7.0 SD (17 months)
pmgnd32468nwl	CCND2 Thr280Asp (P) (51%)	F	BPP ventriculomegaly	Infantile spasms, generalized tonic and myoclonic (6 months)	Macrocephaly OFC +3.1 SD (5 months)
pmgnd40502bikl	DYNC1H1 Arg2244Trp (LP) (55%)	F	BPP	Epileptic spasms, focal epilepsy (13 months)	Acquired microcephaly, OFC 26th %ile (15 months), —2.1 SD (14 years); intellectual impairment (expressive language deficits, no reading)
pmgnd37993pwl	WDR62 Arg512Gly (VUS), Ile594ThrfsTer3 (LP) (42%/51%)	M	BGP	Focal epilepsy (10 years)	Microcephaly OFC −2.05 SD (10 years); severe ID

ACMG, American College of Medical Genetics; BGP, bilateral generalized PMG; BPP, bilateral perisylvian PMG; F, female; ID, intellectual disability; L, left; LP, likely pathogenic; M, male; MRI, magnetic resonance imaging; OFC, occipitofrontal circumference; P, pathogenic; PMG, polymicrogyria; R, right; SD, standard deviation; VAF, variant allele frequency; VUS, variant of unknown significance. All variants are de novo with the exception of the biallelic variants in *WDR6*