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Characterization of mutations of the phosphoinositide-3-kinase regulatory subunit, *PIK3R2*, in perisylvian polymicrogyria: a next generation sequencing study

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Conflicts of Interest

The authors report no conflict of interest.

Web links:

Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [accessed August, 2015].
Freebayes, https://github.com/ekg/freebayes
IGV, http://www.broadinstitute.org/igv/
National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project, http://evs.gs.washington.edu/EVS/
PEAR, http://www.exelixis-lab.org/web/software/pear
Primer3, http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/
Picard, http://picard.sourceforge.net/

Contributors

G.M.M., R.G., and W.B.D designed the study. G.M.M., S.C., V.C. B.M. and D.M. performed the genetic experiments. G.M., V.C., A.R.T., E.A.B., D.M., J-F.D., P.N., J.S. and J.C. analyzed the molecular data. G.M.M., C.D.S., S.A., M.C., S.B., R.B.H., A.G., Y.N-K., C.A., K.J., K.F., K.H., C.H., A.G., E.P., A.S., E.B., C.B., W.B.D., and R.G. recruited and evaluated the study subjects. C.A. provided administrative support and recruited study subjects, G.M.M., R.G., and W.B.D. supervised the study. G.M.M. and R.G. wrote the manuscript.

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SUMMARY

Background—Bilateral perisylvian polymicrogyria (BPP), the most common form of regional polymicrogyria, causes the congenital bilateral perisylvian syndrome, featuring oromotor dysfunction, cognitive impairment and epilepsy. BPP is etiologically heterogeneous, but only a few genetic causes have been reported. The aim of this study was to identify additional genetic etiologies of BPP and delineate their frequency in this patient population.

Methods—We performed child-parent (trio)-based whole exome sequencing (WES) on eight children with BPP. Following the identification of mosaic *PIK3R2* mutations in two of these eight children, we performed targeted screening of *PIK3R2* in a cohort of 118 children with BPP who

were ascertained from 1980 until 2015 using two methods. First, we performed targeted sequencing of the entire *PIK3R2* gene by single molecule molecular inversion probes (smMIPs) on 38 patients with BPP with normal-large head size. Second, we performed amplicon sequencing of the recurrent *PIK3R2* mutation (p.Gly373Arg) on 80 children with various types of polymicrogyria including BPP. One additional patient underwent clinical WES independently, and was included in this study given the phenotypic similarity to our cohort. All patients included in this study were children (< 18 years of age) with polymicrogyria enrolled in our research program.

Findings—Using WES, we identified a mosaic mutation (p.Gly373Arg) in the regulatory subunit of the PI3K-AKT-MTOR pathway, *PIK3R2*, in two children with BPP. Of the 38 patients with BPP and normal-large head size who underwent targeted next generation sequencing by smMIPs, we identified constitutional and mosaic *PIK3R2* mutations in 17 additional children. In parallel, one patient was found to have the recurrent *PIK3R2* mutation by clinical WES. Seven patients had BPP alone, and 13 had BPP in association with features of the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH). Nineteen patients had the same mutation (Gly373Arg), and one had a nearby missense mutation (p.Lys376Glu). Across the entire cohort, mutations were constitutional in 12 and mosaic in eight patients. Among mosaic patients, we observed substantial variation in alternate (mutant) allele levels ranging from 2·5% (10/377) to 36·7% (39/106) of reads, equivalent to 5–73·4% of cells analyzed. Levels of mosaicism varied from undetectable to 17·1% (37/216) of reads in blood-derived compared to 29·4% (2030/6889) to 43·3% (275/634) in saliva-derived DNA.

Interpretation—Constitutional and mosaic mutations in the *PIK3R2* gene are associated with a spectrum of developmental brain disorders ranging from BPP with a normal head size to the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome. The phenotypic variability and low-level mosaicism challenging conventional molecular methods have important implications for genetic testing and counseling.

Keywords

PIK3R2; polymicrogyria; megalencephaly; MPPH syndrome; mosaicism

Introduction

Polymicrogyria is a cortical malformation characterized by excessive gyration and disordered lamination, and is among the most common malformations of cortical development (MCD).[1] Bilateral perisylvian polymicrogyria (BPP) is the most common sub-type of polymicrogyria,[2] and was first reported as a distinct anatomoclinical syndrome in 1993.[3] Many heterogeneous non-genetic and genetic etiologies have been proposed for polymicrogyria, in general, and BPP in particular.[4] Extrinsic non-genetic etiologies include vascular or hypoxemic insults (e.g. twin-twin transfusion syndrome), and congenital cytomegalovirus infection.[1] Genetic causes are collectively rare for BPP and typically occur in clinically recognizable syndromic forms, the most common of which are 1p36.3 and 22q11.2 deletion syndromes. To date, only one gene – *RTTN* – has been associated with isolated BPP in two unrelated families.[5]

The aim of our study was to identify additional genetic causes of BPP. Using whole exome sequencing and targeted sequencing methods, we identified mosaic and constitutional mutations in the *PIK3R2* gene in a subset of children with BPP with normal to large head size.

Methods

Patient Cohort

This study was conducted at the Seattle Children's Research Institute (SCRI) and the University of Florence Meyer's Children's Hospital. Patients at both centers were enrolled in the developmental brain disorders research program. Patients included in this study were children less than 18 years of age with polymicrogyria identified by brain imaging, with or without brain overgrowth (or megalencephaly). Patients with inadequate imaging and/or clinical data were excluded from this study. Informed written consent was obtained from all of the patients' legal guardians to share clinical, neuroimaging and electroencephalographic (EEG) data, as well as provide key research samples including blood, saliva, and skin, when available. Clinical and neuroimaging studies were reviewed by the investigators. This study was approved by the Seattle Children's Institutional Review Board (IRB) and the Pediatric Review Board of the Tuscany Region.

Magnetic resonance imaging

A comprehensive MRI investigation was performed in every patient, using different imaging systems including either 1·5-, 3- or 7-Tesla scans. Minimal sequences requirement consisted of noncontrast-enhanced spin echo, inversion recovery, and gradient echo sequences performed in the axial, sagittal, and coronal planes. All patients were examined with 5-mm or lower slice thickness. The ultra high-field 7-Tesla MRI included 3D-T1 weighted fast-spoiled gradient echo (FSPGR), 3D susceptibility-weighted angiography (SWAN), 2D T2*-weighted targeted dual-echo gradient-recalled echo (GRE), 2D T2-weighted DSE and 2D grey-white matter tissue border enhancement (TBE) FSE-IR.

Molecular methods

Genomic DNA was extracted from patients' tissues using standard protocols using the Qiagen Puregene Blood Core Kit with RNase for blood, and the Oragene Saliva Kit following the manufacturers' recommendations. First, DNA samples from eight child-parent trios with BPP were subjected to whole exome sequencing (WES). Patients selected for WES were those for whom an underlying genetic cause has not been identified by prior standard testing that includes a chromosomal microarray, and who have no clinical or imaging findings suggestive of a non-genetic etiology. Of these eight patients, two had megalencephaly (defined as occipito-frontal circumference, OFC, > 2 standard deviations, SD, above the mean for age and gender), two had borderline small head size (OFC 2 or more SD below the mean for age and gender), and the remaining four were normocephalic. The parents of all eight children were clinically unaffected. Mean occipito-frontal circumference measurements and standard deviations for age and sex were calculated using the standard Nellhaus Head Circumference Charts for children from birth to 18 years.[6]

To further assess the frequency of the *PIK3R2* mutation, p.Gly373Arg, that was seen in two of our patients who underwent whole exome sequencing (and was therefore considered, recurrent), we developed an allelic discrimination (AD) assay to screen a cohort of 80 children with polymicrogyria broadly (without using head size as a selection criteria). The presence of two primer/probe pairs marked with two different fluorescent dyes in the same AD assay allowed us to assess the allelic status at the mutation site. In parallel, we screened 38 patients using single molecule molecular inversion probes (smMIPs) for mutations in *PIK3R2*.[7] These 38 patients had BPP in association with either a normal head size (N = 6) or large head size (N = 32). An additional patient with features of the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) underwent clinical trio-based WES independently.

WES analysis—Library preparation, exome enrichment and WES were performed at the French National Centre for Genotyping (CNG, Evry, France). Libraries were prepared from 3 μg genomic DNA extracted from whole blood using an optimized SureSelect Human Exome kit (Agilent). Captured, purified and clonally amplified libraries targeting the exome were then sequenced on a HiSeq 2000 (Illumina). Sequence reads were aligned to the human genome (hg19 assembly) using BWA software. Downstream processing was carried out with the Genome analysis toolkit (GATK), SAMtools and Picard Tools. Single-nucleotide variants and indels were subsequently called by the SAMtools suite (mpileup, bcftools, vcfutil). All calls with a read coverage ≤5× and a Phred-scaled SNP quality of ≤20 were filtered out. Substitution and variation calls were made with the SAMtools pipeline (mpileup). Variants were annotated with an in-house Paris Descartes bioinformatics platform pipeline based on the Ensembl database (release 67). Exome sequencing quality data were homogeneous with an average mean depth higher than 100X. Coverage depth greater than 15X and 5X were obtained for ~97% and ~99% of the target. We analysed variants affecting coding regions and essential splice sites and excluded all variants with frequencies higher than 1% in multiple genome databases including dbSNP, 1000 Genomes, the NHLBI Exome Variant Server (EVS), the Exome Aggregation Consortium (ExAC), and a local Paris Descartes Bioinformatics platform database.

Multiplex targeted sequencing using smMIPs.[7]—We designed a pool of 35 smMIP oligonucleotides targeting the coding sequences of *PIK3R2*. smMIPs were tiled across a total of 3340 base pair (bp) of genomic sequence, including all 2202 coding nucleotides of the targeted genes. 100 ng capture reactions were performed in parallel. Massively parallel sequencing was performed using the Illumina HiSeq. Variants were filtered against the public databases (dbSNP, 1000 Genomes, EVS, ExAC) mentioned above. smMIP sequencing data was processed with MIPgen and PEAR 0.8.1,[8] both with default options, with the exception of introducing a penalty of 80 for soft clipping during the BWA mem mapping, to produce high quality smc-reads (single molecule consensus reads). smc-reads were analyzed with GATK v3.1–1 as recommended using the IndelRealigner and HaplotypeCaller tools on the targeted regions. smc-reads were processed with Freebayes using the -F 0 option to capture low frequency variants. All variants with at least two reads were retained for downstream analysis. Variants were merged across all samples and allele balances calculated.

Amplicon sequencing—To screen for the recurrent *PIK3R2* mutation, p.Gly373Arg, we performed locus-specific amplification of genomic DNA followed by GS Junior sequencing. We designed fusion primers containing genome-specific sequences along with distinct MIDs (multiplex identifier sequences) used to differentiate samples being run together on the same plate and sequencing adapters to generate amplicons ranging in size from 290 to 310 bp using primer3plus software. Primer sequences are available upon request (Dr. Renzo Guerrini). Small DNA fragments were removed using Agencourt AMPure XP (Beckman Coulter, Beverly, MA) according to the manufacturer's protocol. All amplicons were quantified using the Quant-iT PicoGreen dsDNA reagent (Invitrogen Corporation, Life Technologies, Carlsbad, CA), pooled at equimolar ratios, amplified by emulsion PCR using the GS Junior Titanium emPCR kit (Lib-A kit, Roche Applied Science, Mannheim, Germany) and pyrosequenced in the sense and antisense strands on a GS Junior sequencer (Roche) following the manufacturer's instructions. We performed data analysis using the GS Amplicon Variant Analyzer version 3.0 (AVAv3.0) software (Roche).

Sanger sequencing—We performed confirmation of constitutional mutations by direct Sanger sequencing. PCR amplification was performed with 50 ng of genomic DNA using Taq DNA polymerase (Applied Biosystems). Primers used to amplify the coding and flanking noncoding regions of *PIK3R2* were designed using Primer 3. Double-stranded DNA sequence analysis was performed using the Big Dye Terminator chemistry (Applied Biosystems), and reactions were run on the ABI 3730_l Genetic Analyzer (Applied Biosystems). Sequence chromatograms were analyzed using Mutation Surveyor software version 3.30. Sequences were compared with normal control samples and the reference sequences for *PIK3R2*.

Statistical analysis—P-values were calculated by using Fisher's exact test. 95% confidence intervals were calculated by using the method introduced by Newcombe.[9]

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Results

Sporadic unexplained cases of BPP are by far among the most frequent conditions of the heterogeneous group of MCD. In order to further delineate the contribution of genetic

causes corresponding to *de novo* mutation events, we selected eight child-parent trios. All families tested by WES had a single affected patient (sporadic case) with BPP. Figure 1 highlights our overall experimental workflow for detecting and prioritizing sequence variants and the validation methods of our molecular findings. This workflow is an adaptation of the one we have previously used to search for MCD-related genes.[10,11] In line with previous studies we identified approximately 7000 variants in each exomed individual and an average of 245 variations per subject after filtering.[10,12,13] Supplementary Tables 1 and 2 provide data on WES quality metrics, as well as *de novo variants* identified in this cohort, respectively.

Filtering of exome data and search for variations in the same gene in unrelated subjects revealed the same recurrent mutation (c.1117G>A, p.Gly373Arg), in *PIK3R2* in two patients (Patients 18 and 19). This mutation was not present in any of the public databases. However, close look at the reads generated by the high throughput sequencing using the Integrated Genome Viewer (IGV) interface revealed that this variant is present in 10 reads out of 86 (12%) for patient 18 and 20 reads out of 132 (15%) for patient 19. This deviation from 50% of reads bearing the variant or alternate allele expected for heterozygous constitutional mutations was suggestive of somatic mosaicism of this mutation in *PIK3R2*. As with standard WES, variation in read depth between DNA samples is due to quantity and quality of the initial DNA, efficiency of DNA binding to target, amplification of the final library and clusters, and sequencing efficiency. To further confirm and quantify the suspected somatic mosaicism, we performed deep targeted sequencing of the coding sequences of *PIK3R2* using DNA extracted from blood and saliva of these two patients by Amplicon sequencing, which showed variable mutation levels in both patients among tissues tested, confirming somatic mosaicism.

Given the identification of a recurrent mosaic mutation (p.Gly373Arg) in *PIK3R2* in BPP, which is also the same mutation identified previously in MPPH[14], we sought to search for mutations in this gene in a cohort of 118 patients with polymicrogyria. Thirty-eight had BPP with normal or large head size (including 32 with MPPH) and were tested by smMIPs and Sanger Sequencing. This testing strategy identified mutations in 17 patients, 16 of whom were found to have the same *PIK3R2* mutation identified by WES (p.Gly373Arg). Another patient with MPPH (patient 5) was independently studied by clinical WES and found to have the same *PIK3R2* mutation as well. One patient was identified to have a *de novo* missense mutation within the same functional domain of the *PIK3R2* gene, p.Lys376Glu. Eighty additional patients with polymicrogyria broadly were tested only for the recurrent *PIK3R2* mutation (c.1117G>A, p.Gly373Arg) by amplicon sequencing and were found to be negative. The clinical characteristics of all patients included in this study are summarized in Table 1.

As the same *PIK3R2* mutation was detected in a subset of patients with polymicrogyria among a cohort of 126, we calculated the probability for the recurrent *PIK3R2* mutation occurring by chance in our cohort. Comparing the allele frequency of the *PIK3R2* nonsynonymous variant in our cohort (19/(126 • 2)); with the one reported in the largest public database (ExAC; 0/33,113) showed an overwhelming significant enrichment of

PIK3R2 variant in our cohort using Fisher's exact test (p-value < 2.2×10^{-16}) (Supplementary Tables 3–6).

Across the cohort, mutations were constitutional in 12 and mosaic in eight patients. Among the mosaic patients, we observed substantial variation in alternate (mutant) allele levels within individual samples, ranging from 2·6 (10/377) to 36·7% (39/106) of reads, equivalent to 5·2–73·4% of cells analyzed. Levels of mosaicism varied from undetectable to 17·1% (37/216) of reads in blood-derived compared to 29·4 (2030/6889) to 43·3% (275/634) in saliva-derived DNA. To exclude artifactual low frequency variant detection due to sample cross-contamination or index cross talk, we confirmed mutations using independent captures or Sanger sequencing. Patient 12 had a different de novo missense mutation of *PIK3R2* (c. 1126A>G, p.Lys376Glu), that was not present in any of the public databases and is predicted to be pathogenic using in silico analysis. This *de novo* mutation also affects a highly evolutionarily conserved amino acid residue within the SH domain of PIK3R2, and is therefore predicted to be pathogenic.[15] The clinical-neuroimaging and molecular findings of our *PIK3R2* mutation-positive patients are summarized in Tables 2 and 3, respectively. Representative brain MRI images for patients with constitutional and mosaic mutations are shown in Figures 2 and 3, respectively. All patients had BPP, with or without megalencephaly. Below, we summarize the most distinctive phenotypic characteristics of these patients which include polymicrogyria, megalencephaly, ventriculomegaly, epilepsy, and oromotor weakness.

Polymicrogyria only affected the perisylvian cortex or extended beyond it with perisylvian predominance. The severity spectrum ranged from BPP restricted to the posterior perisylvian regions *(grade 4)* to BPP involving the entire perisylvian regions *(grade 3)*, to BPP extending variable distances anteriorly, posteriorly, and inferiorly from the perisylvian regions but sparing the occipital and frontal lobes *(grade 2)*, to extensive BPP that includes one or both poles with the Sylvian fissures extended posteriorly and often oriented superiorly (*grade 1*).[16] The extent of involvement was bilateral, but often mildly asymmetric in most individuals.

Thirteen of 20 (65%) of individuals in our cohort had megalencephaly defined as OFC > 2 standard deviations (SD) above the mean for age and gender, fulfilling the diagnostic criteria for MPPH.[17] MEG was predominantly congenital in onset in these individuals; with later OFCs reported as large as 7·5 SD above the mean. 7/20 (35%) individuals were normocephalic.

Ventriculomegaly, ranging from mild to severe, was seen in 17/20 (85%) individuals, including one with hydrocephalus requiring neurosurgical intervention (by placement of a ventriculostomy drain) The corpus callosum appeared thin or stretched in some of these individuals.

Other neuroimaging abnormalities seen in our cohort include a variably thick corpus callosum (7/20; 35%), cerebellar tonsillar ectopia (5/20; 25%), mild white matter dysmyelination with prominent perivascular spaces (7/20; 35%), and cavum septum pellucidum et vergae (5/20; 25%).

Epilepsy occurred in 14/20 (70%) individuals. Seizure onset ranged from 1 month to 12 years of age (with a mean age of onset of 2 years and 3 months across the entire cohort, except for patients 1 and 15 for whom age of seizure onset was unknown). Seizures were predominantly focal, although no clearly recurrent seizure pattern emerged. Although epilepsy was a prominent clinical feature, it was the reason for first referral in a minority of patients. In that subset, it manifested with severe, intractable seizures, including one patient who had infantile spasms that evolved into myoclonic seizures. Overall, severe epilepsies were most often seen in patients with constitutional mutations, who also had an overall earlier age at seizure onset (mean 11 months vs. 3·89 years for patients with constitutional vs. mosaic mutations, respectively).

Symptoms of oromotor dysfunction such as expressive language or speech delay, difficulties handling oral secretions (such as profuse drooling) and dysphagia were present in the majority of our patients (9/12; 75%; of patients with constitutional mutations and 7/8; 87·5%; of patients with mosaic mutations).

Other notable manifestations included cutaneous capillary malformations (seen in four patients), and multiple ventricular septal defects (seen in one patient). Two patients had hypoglycemia. In one (patient 5), it was transient at birth. The other (patient 14) had atypical ketotic hypoglycemia at six years of age. All of the patients in our series had intellectual disability that varied from mild to severe. One patient with MPPH (patient 17) exhibited early severe autistic features.

Constitutional *PIK3R2* mutations were *de novo*, with the exception of two families. The first family (of patient 7) consists of a large sibship of 11 children from multiple fathers, of whom five have megalencephaly, BPP and variable hydrocephalus. One of these five children also had postaxial polydactyly, a known feature of MPPH. The mother has macrocephaly, hydrocephalus, intellectual disability, epilepsy and schizoaffective disorder, but no brain imaging was available. Both child and mother harbored the *PIK3R2* mutation in peripheral blood-derived DNA at mutant allele levels of 47% (23/48) and 41% (33/80) of reads, respectively, suggestive of maternal inheritance. Samples were not available from the other affected children. The second family (of patients 10 and 11) consists of two affected siblings (boy and girl) with congenital megalencephaly, BPP, mild ventriculomegaly, epilepsy and intellectual disability. Both siblings also had cutis marmorata. Parental testing of blood-derived DNA was negative by deep targeted sequencing, suggestive of parental germline mosaicism. The pedigrees of these families are shown in the Supplementary Figure.

Discussion

In this study, we report *PIK3R2* mutations in 20 children including 13 with MPPH syndrome and seven with BPP without megalencephaly. *PIK3R2* mutations identified in our cohort include *de novo* constitutional mutations, mutations inherited from an affected parent or from parental germline mosaicism, as well as mosaic mutations. Our results show that mutations of this gene are associated with a spectrum of malformations of cortical

development ranging from isolated BPP with a normal head size to BPP with megalencephaly, including the MPPH syndrome (Research in context).

Bilateral perisylvian polymicrogyria (BPP) is the most common subtype of polymicrogyria and has been proposed to be an etiologically heterogeneous anatomoclinical syndrome, featuring a combination of oromotor dysfunction, cognitive impairment and epilepsy.[1, 18,19] Among the genetic causes, BPP has most often been reported in individuals with copy number variants, especially 1p36.3 and 22q11.2 deletion syndromes.[20,21] However, genetic heterogeneity has been proposed based on reports of large families with possible autosomal dominant or X-linked inheritance with incomplete penetrance.[19, 22] Polymicrogyria of variable severity and distribution has been reported in many brain malformation syndromes caused by mutations in a growing number of genes including *NDE1, WDR62, OCLN, RAB3GAP1, RAB3GAP2, RAB18, DYNC1H1, KIF5C, EOMES, RTTN, FH* and *KIAA1279*, as well as many of the tubulin genes (*TUBA1A, TUBA8, TUBB2B, TUBB3, TUBB*).[1] However, only for the *TUBA1A, TUBB2B* and *OCLN* genes has polymicrogyria been neuropathologically demonstrated.[23–25] For malformation syndromes related to the remaining genes, the defining characteristics of polymicrogyria, which are typically microscopic (multiple small microgyri, formed by thinned cortex, fused together) have been inferred based on the macroscopic appearance of the gyral pattern, as visible by MRI (gyri of irregular size and shape, cortical infolding and thickening related to fused microgyri). However, the underlying architerctural substrate and developmental mechanisms might vary in the different polymicrogyria syndromes, in spite of similar imaging features.

Mutations of genes within the phosphatidylinositol-3-kinase (PI3K)-AKT-MTOR pathway are known to cause a wide spectrum of developmental brain and body disorders. Specifically, mutations of *PIK3CA, PIK3R2, PTEN, AKT3* and *CCND2* have been associated with focal, segmental (multifocal) and generalized megalencephaly (MEG) with variable other features (Supplementary Table 7).[14,26–28] *PIK3R2* mutations specifically cause the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH),[14,17] a relatively rare developmental brain disorder characterized by megalencephaly, polymicrogyria, ventriculomegaly often leading to hydrocephalus, and postaxial polydactyly. [17, 29–32] To date, mutations of *PIK3R2* have been reported in 15 individuals with this syndrome.[14,33,34] Mutations in two additional core pathway genes – *AKT3* and *CCND2* – have recently been associated with MPPH as well.[14,28] While mutations in PI3K-AKT-MTOR pathway genes such as *PIK3CA* have been predominately post-zygotic or mosaic, mutations of *PIK3R2, AKT3* and *CCND2* have been predominantly *de novo* constitutional, with only one *PIK3R2* mosaic mutation reported to date.[35]

Our data show that mutations of *PIK3R2* are an important cause of BPP, which otherwise remains etiologically heterogeneous. Overall, constitutional and mosaic *PIK3R2* mutations accounted for 15% (19/126) of our cohort of patients with polymicrogyria, with mosaic mutations accounting for (8/126) 6·3% of the cohort. This rate is higher than that observed in most MCD.[1, 35] Epilepsy was a prominent clinical feature. Although no association with particular epilepsy syndromes was apparent, an earlier age at seizure onset and more severe epilepsy outcomes were also observed in patients with constitutional mutations.

PIK3R2 encodes the p85β regulatory subunit of the PI3K-AKT-MTOR pathway. The mutational spectrum is very narrow as all but one of reported patients harbored the same missense mutation, p.Gly373Arg. This gain of function mutation lies within the sequence homology (SH) domain of the gene and is seen infrequently in somatic tissues in cancer.[36] Our data therefore expand on the phenotypic spectrum of *PIK3R2* mutations, reporting the first *PIK3R2* mutations in BPP alone without other features of MPPH syndrome. We also report a second mutation of *PIK3R2* (p.Lys376Glu) in a girl who has BPP.

Mutations of other upstream (*PTEN, PIK3CA*), central (*AKT3, TSC1, TSC2*) and downstream (*CCND2*) genes within the PI3K-AKT-MTOR pathway are also associated with a wide range of developmental brain disorders. The phenotypic spectrum of brain involvement ranges from bilateral diffuse megalencephaly with normal gyral pattern to megalencephaly with polymicrogyria to hemimegalencephaly to focal cortical dysplasia (FCD) type 2 (Supplementary Table 7).[14, 26–28, 37–38]

Our findings show that mosaic mutations of *PIK3R2* cause a regional brain malformation, similar to our experience with *PIK3CA*.[14] While the level of mosaicism partly explains the variable severity, the basis of the perisylvian predominance is not known. Bearing in mind the limited sensitivity of MRI investigations, we hypothesize that the perisylvian region is more vulnerable to perturbations caused by *PIK3R2* mutations, even when occurring in a limited number of randomly distributed cells. The primary fissure first appears as a depression from the 5th intrauterine month and completes opercularization after birth.[39] The closure of the frontal and temporal opercula over the insula is among the most complex morphological changes occurring in the postembryonic cerebral hemispheres.[40] Deviations in cortical growth due to increased cell proliferation or impaired microvascular development, both likely to occur with *PIK3R2* mutations, might interfere with the dynamics and cytoarchitectural determinants that generate the pattern of cortical folding in the perisylvian region.[39,41] However, it remains difficult to determine to what extent a regional brain malformation such as perisylvian polymicrogyria results from enhanced local vulnerability due to altered dynamics of cortical development or just reflects the regional expression of the mutant gene.

While our exome analysis pipeline allowed the detection of mosaic mutations in two of our BPP patients, it is possible that other mosaic mutations in this cohort were missed due to either poor coverage or very low level of mosaicism. We speculate this is unlikely as the average depth of coverage across our exomes is 141X and full coverage of *PIK3R2* coding exons was checked for our eight trios. Further, as we used a site-specific method (amplicon sequencing) to efficiently screen our cohort of 80 patients with polymicrogyria, we may have missed other mutations within the *PIK3R2* gene in this group. One additional potential limitation with respect to findings described in this report is that the study is based mainly on analysis of DNA extracted from peripheral tissues (blood, saliva), and brain tissues were not accessible to detect or confirm mosaic mutations. We expect that future NGS studies of additional patients will further delineate the frequency of *PIK3R2* mutations in polymicrogyria in general, and BPP in particular. Finally, our study similar to others expands the number of families with possible germline mosaicism. The role of germline mosaicism (i.e. mosaic mutations in the germline cells of a parent) is increasingly being

recognized as the cause of genetic disorders. [42–43] We anticipate that the frequency of germline mosaicism in the *PIK3R2* related spectrum in particular will be further delineated with future NGS studies as well.

In summary, our report shows that both constitutional and mosaic mutations of *PIK3R2* cause a spectrum of developmental brain disorders, similar to several other PI3K-AKT-MTOR pathway genes. In addition, we report the second pathogenic mutation of this gene, the second family with probable parental germline mosaicism, and the first evidence of parent-child transmission of MPPH. These data have important implications for familial testing and recurrence risk counseling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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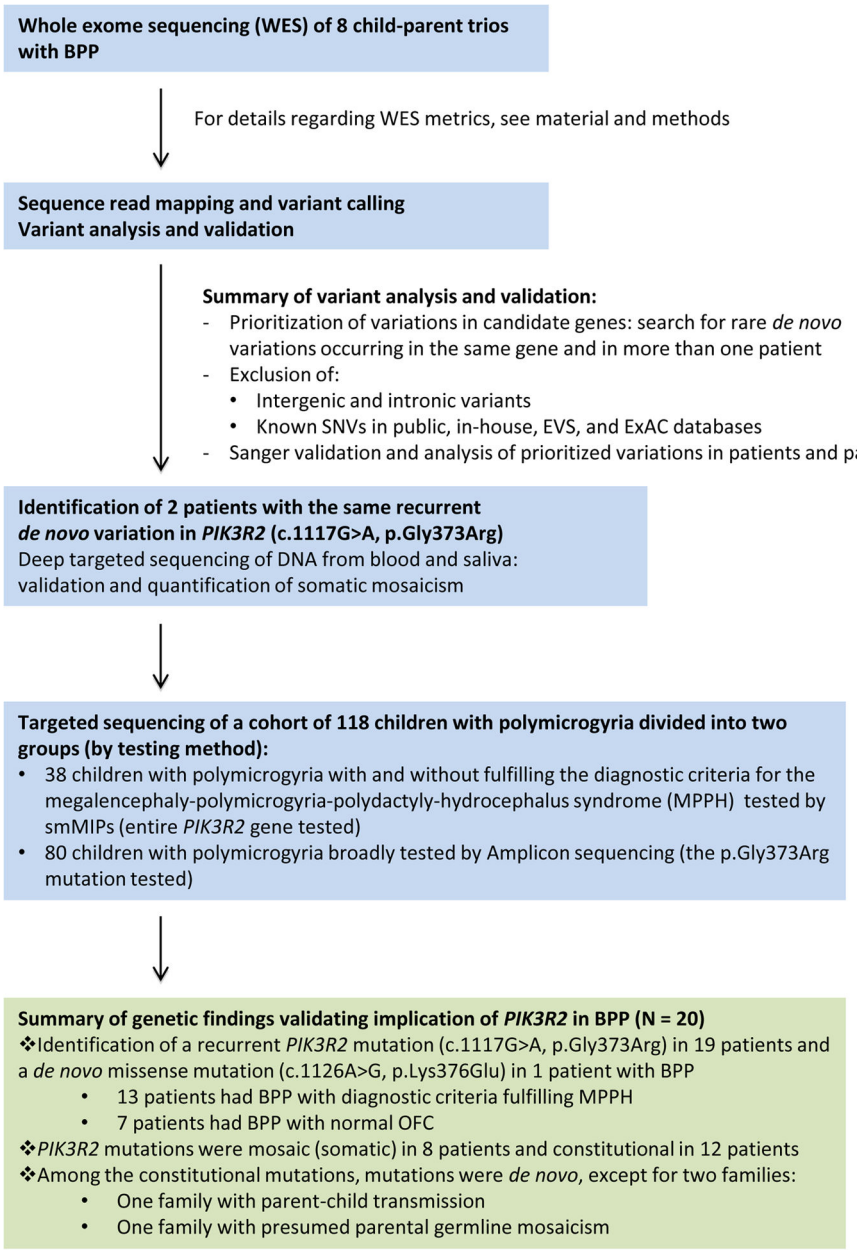

Figure 1. Experimental workflow of this study that allowed detection of the *de novo* sequence variation in *PIK3R2* gene in individuals with BPP

The entire exome sequencing methodology and workflow used in this study are adaptations of those previously reported in Poirier et al (2013).[10]

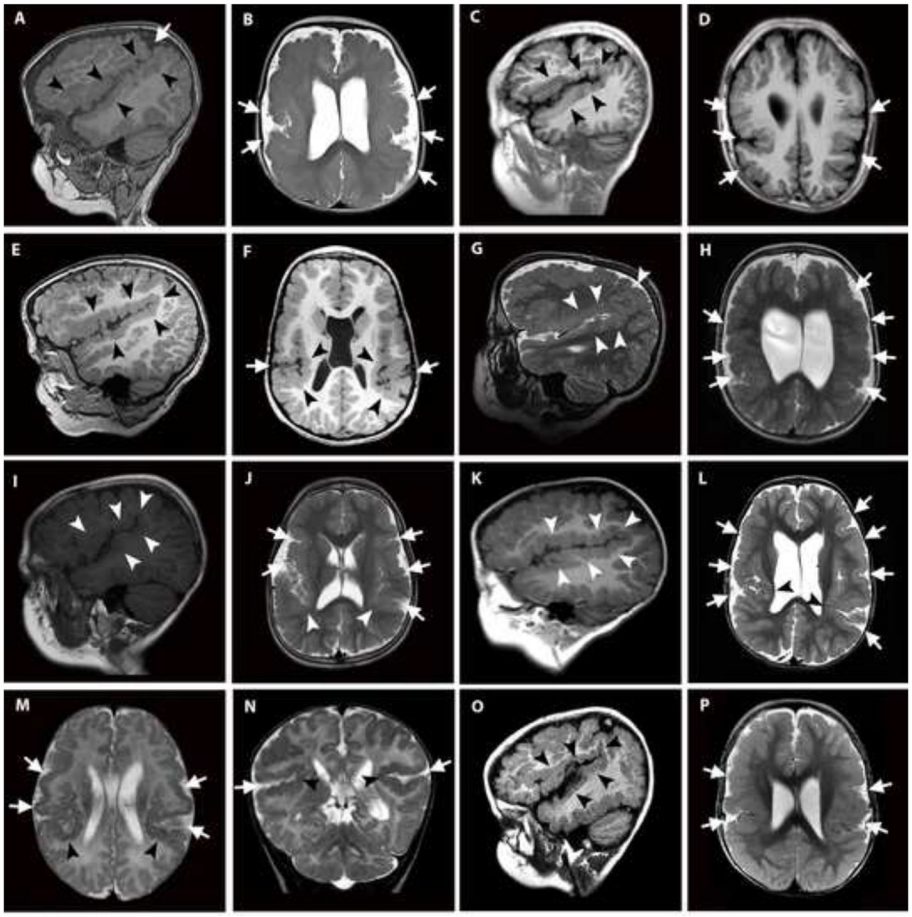

Figure 2. Brain MRI images of patients with constitutional *PIK3R2* mutations

Representative T1 and T2-weighted mid-sagittal, axial and coronal 3 Tesla (T) brain MRI images in patients 2 (LR12-099) at age two years (A, B), 3 (LR12-415) at age eight years (C, D), 5 (LR13-242) at age five years (E, F), 6 (LR13-398) at age three years (G, H), 7 (LR08-305) at age two years (I, J), 9 (LR13-088) at age one year and six months (K, L), 11 (LR13-157a2) at age 21 days (M, N) and 12 (LR08-308) at age five years (O, P). Note bilateral perisylvian polymicrogyria (BPP) (arrows), and superiorly extended sylvian fissures (arrowheads). Other notable features include moderate to severe ventriculomegaly (B, F, H, L, P), and cavum septum pellucidum et vergae (F, J and M). White and black arrowheads are used interchangeably to contrast with the background.

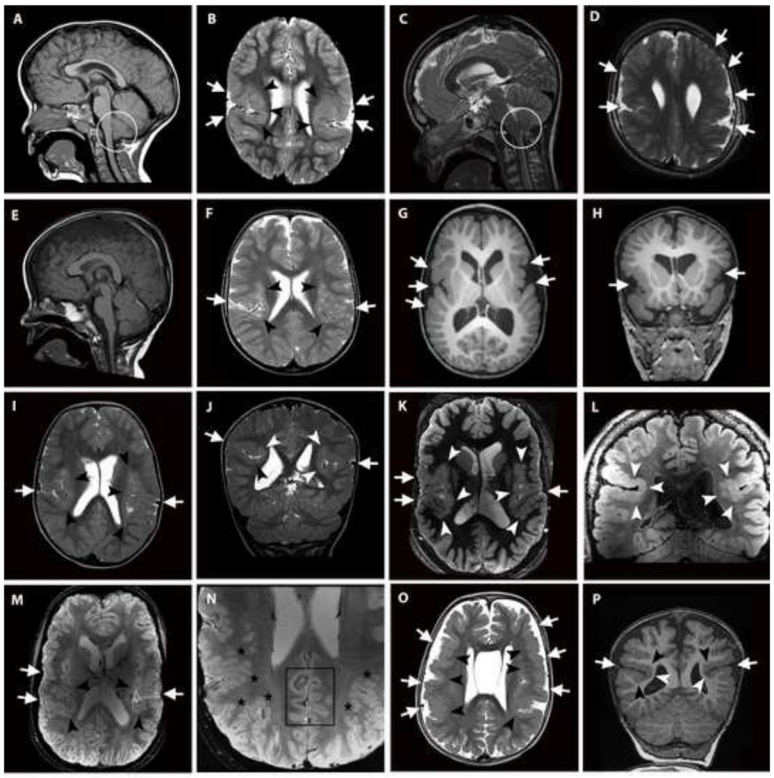

Figure 3. Brain MRI images of patients with mosaic *PIK3R2* mutations

Representative T1 and T2-weighted, SWAN, IR, 3T and 7T mid-sagittal, axial and coronal brain MRI images in patients 13 (LR09-216) at age four years (A, B), 14 (LP99-083) at age 12 years (C, D), 15 (LR11-322) at age two years (E, F), 16 (LR13-409) at age three years (G, H), 17 (LR13-302) at age two years (I, J), 18 (1734P) at age 14 years (K, L), 19 (1317N) at age 22 years (M, N) and 20 (LR11-278) at age 3 years (O, P). Note bilateral perisylvian polymicrogyria (BPP) (arrows), and extended sylvian fissures (arrowheads). Images K, L, M and N are at 7T. Note in image N the different morphological pattern between the normal mesial parieto –occipital cortex (square) and the undulated packed and infolded microgyri in the lateral parietal cortex (asterisks). Other notable features include mild-moderate ventriculomegaly (G, H, I, J, K, L, M, O), cerebellar tonsillar ectopia (A, C) (white circles), thick corpus callosum (C, E), and cavum septum pellucidum et vergae (G,H, O). White and black arrowheads are used interchangeably to contrast with the background.

Table 1

Summary of the clinical and neuroimaging features of the cohort included in this study (N=127)

	Mutation-positive patients		Mutation-negative patients		
Cohort/Feature	**Constitutional *PIK3R2* mutations (N=12)**	**Mosaic *PIK3R2* mutations (N=8)**	**BPP WES (N=6)**	**PMG-Amplicon Sequencing (N=80*)**	**BPP smMIPs (N=21)**
Gender	8 F, 4 M	4 F, 4 M	4 F, 2 M	30 F, 23 M	8 F, 13 M
Ethnicity	11/12 Caucasian 1/12 African-American	8/8 Caucasian	6/6 Caucasian	53/53 Caucasian	11/21 Caucasian, 2/21 African American, 2/21 Asian, 2/21 Hispanic, 4/21 Unknown
OFC Measurements					
Mean OFC (in SD) at birth for females/males	3·8/4·8	4/3·1	0/0	1/0	1·6/3·33
Mean OFC (in SD) at last assessment for females/males	3·75/5·25	2·8/4·7	0/2	1·2/0·7	4·25/3·2
Age range of last assessment	14mo–8·5yrs/3mo–18yrs	7mo–22yrs/4mo–14yrs	4·5yrs–16yrs/3,7yrs	3mo–13·5 yrs/1–15·5yrs	5·5mo–7·5yrs/13mo–7·5yrs
Megalencephaly (OFC >2 SD)	9/12 (75%)	7/8 (88%)	0/6 (0%)	2/53 (4%)	19/21 (90%)
Brain Imaging					
Polymicrogyria (BPP) Grade 1–2	10/12 (83%)	6/8 (75%)	3/6 (50%)	24/53 (45%)	5/21 (24%)
Polymicrogyria (BPP) Grade 3–4	2/12 (17%)	2/8 (25%)	3/6 (50%)	29/53 (55%)	16/21 (76%)
Ventriculomegaly	12/12 (100%)	5/8 (63%)	0/6 (0%)	6/53 (11%)	9/21 (43%)
Hydrocephalus (s/p shunting)	1/12 (8%)	0/8 (0%)	0/6 (0%)	0/53 (0%)	3/21 (14%)
Thick corpus callosum	5/12 (42%)	3/8 (38%)	0/6 (0%)	2/53 (4%)	4/21 (19%)
Epilepsy	7/12 (58%)	6/8 (75%)	1/6 (17%)	37/53 (70%)	13/21 (62%)
Mean age of seizure onset	11 mo	3·89 yrs	2·25 yrs	3·35 yrs	14 mo
SD	8·58 mo	4·74 yrs	NA	3·85 yrs	9·92 mo
Oromotor weakness	9/12 (75%)	7/8 (88%)	4/6 (67%)	13/53 (25%)	10/21 (48%)

Abbreviations: BPP = bilateral perisylvian polymicrogyria; F = female; M = male; mo = months; OFC = occipito-frontal circumference; PMG = polymicrogyria; SD = standard deviations; smMIPs = single molecule molecular inversion probes; WES = whole exome sequencing; yrs = years.

*Of these 80 patients, clinical data were available on 53 patients. However, all 80 patients were confirmed to have polymicrogyria by assessment of their neuroimaging.

Table 2

Summary of the clinical features and neuroimaging features of patients harboring *PI3KR2* mutations (N=20)

N	DB#	Sex	Age	OFC (cm) at birth	OFC (cm) at last assessment	Polymicrogyria	Additional brain abnormalities	Reason of first medical evaluation (age)	Epilepsy (onset)	Neurological examination	Oromotor weakness	Cognitive level	Other clinical features
Patients with the constitutional c.1117G>A, p.Gly373Arg *PIK3R2* mutation													
1	LR11-321	F	2·5 yrs	44 (+7·5 SD)	59 (+7·5) at 2.5 yrs	BPP grade 1–2	Moderate ventriculomegaly, dysmyelination	Macrocephaly (birth)	Epilepsy, no details available	No details available	No details available	Significant LD, global developmental delay	–
2	LR12-099	F	3 yrs	43 (+7)	55.5 (+4) at 3·5 yrs	BPP grade 1–2	Moderate ventriculomegaly, thick CC, thin WM	Progressive macrocephaly (3 months)	No seizures	Quadriparesis	Dysphagia, speech delays (non-verbal; mimics sounds), excessive drooling	Severe ID, poor head control, wheel-hair bound,	GI malrotation, laryngomalacia
3	LR12-415	M	18 yrs	40 (+5)	63·6 (+6) at 18 yrs	BPP grade 1–2	Moderate ventriculomegaly, thick CC	Macrocephaly and hypotonia (infancy)	Rare focal seizures with unresponsiveness (2 yrs)	Hypotonia	Non-verbal	Severe ID (IQ <35), walked with assistance at 4 yrs	Divergent strabismus
4	LR12-303	F	14 mo	39 (+3)	47·7 (+1–2) at 14 mo	BPP grade 3–4	Prominent PV in BG, mild Ventriculomegaly, thin CC, mild CBTE, CSPV, hippocampal dysgenesis	Eye deviation, PMG on MRI (shortly after birth)	None	Hypotonia, hypokinesia	Poor suck/swallow, poor swallow coordination, status post G-tube, excessive drooling, markedly delayed speech	Severe ID	Hyperopia, severe astigmatism, GERD
5	LR13-242	M	5 yrs	41·9 (+5·5)	58 (+6) at 5 age	BPP grade 3–4	Moderate ventriculomegaly, thin CC, prominent PV spaces, CSPV	Macrocephaly, ventriculomegaly detected on prenatal US	None	Normal	Speech delay	Mild-mod ID, walked at 12 mo, fine motor delays	Attention deficit, sensory processing issues, LGA, transient hypoglycemia at birth
6	LR13-298	F	8 yrs 7 mo	No details available	59·5 (+6) at 8·5 yrs	BPP grade 1–2	Hydrocephalus status post ventriculostomy (10 months), CBTE (1–5 mm), stretched CC, thin WM	Ventriculomgaly on prenatal US (in utero, GA 34 weeks)	Infantile spasms evolved into myoclonic seizures (1 yr), intractable	Axial hypotonia, appendicular hypertonia	Dysphagia, dysarthria, profuse drooling	Severe ID, non-ambulatory, non-verbal, no social/ communication skills *	Connective tissue laxity, GERD, short stature at 8 yrs
7	LR08-305[a]	M	6 yrs	No details available	57 (+4–5) at 3 yrs	BPP grade 1–2	Mild ventriculomegaly, mild CBTE (1–3 mm), mildly thick CC	Eye deviation (1 week), macrocephaly (3 months)	Focal seizures with unresponsiveness (1 yr)	Hypotonia, oculomotor apraxia	Delayed speech	Mod ID. Walked at 16 mo, delayed fine motor skills, 80 words at 6 yrs	GERD, dysmorphic facial features[b]
8	LR12-319	F	4 yrs	39 (+3)	55 (+3·5) at 5 yrs	BPP grade 1–2	Severe ventriculomegaly, thin/stretched CC	Macrocephaly, multiple muscular VSDs (birth)	Focal seizures with unresponsiveness (1 mo)	Hemiparesis	Dysphagia with fatigue with food	Moderate ID, walked at 2.5 yrs, speech delay	Multiple VSD, esotropia, hyperopia, astigmatism, broad thumbs, sandal gap toes
9	LR13-088	F	2 yrs	No details available	52 (+4) at 16 mo	BPP grade 1–2	Moderate ventriculomgaly, CBTE (1–5 mm), mildly thick CC	Developmental delays (6 mo)	None	Hemiparesis,	Expressive speech delay, increased drooling	Mild ID	None
10	LR13-157a1	F	8.5 yrs	38 (+2)	55·5 (+2) at 8·5 yrs	BPP grade 1–2	Ventriculomegaly	Seizures commencing at 15 months	Focal seizures with unresponsiveness (15 mo)	Normal	None	Developmental delay at 2 yrs, behind peers, IQ not formally assessed.	Cutis marmorata, Wt +2 SD
11	LR13-157a2	M	4 yrs	40.5 (+4–5)	47·5 (+4–5) at 4 mo	BPP grade 1–2	Mild ventriculomegaly	Macrocephaly identified on prenatal ultrasound; first seizure at 7 weeks	Focal seizures with unresponsiveness (2 mo), intractable	Cortical blindness, otherwise normal neurological examination	None	Mild developmental delay. At 21 mo: gross motor 15 mo, speech 12 mo, fine motor 9 mo, social 9 mo	Cutis marmorata, 1 cutaneous hemangioma
Patient with the constitutional c.1126A>G, p.Lys376Glu *PIK3R2* mutation													
12	LR08-308	F	5 yrs	35 (+0–1 SD)	52·3 (+1–2) at 5 yrs	BPP grade 1–2	Mild ventriculom galy, mildly thick CC	Macrocephaly (3 months)	None	Hypotonia	Expressive language delays, early dysphagia	Moderate ID, walked at 4 yrs. BSID at 10 mo: cognition 3 mo, fine motor 2 mo, social-emotional 4 mo, language (receptive/e xpressive 3 mo, motor 3–4 mo	Cutaneous capillary malformation
Patients with the mosaic c.1117G>A, p.Gly373Arg *PIK3R2* mutation													

N	DB#	Sex	Age	OFC (cm) at birth	OFC (cm) at last assessment	Polymicrogyria	Additional brain abnormalities	Reason of first medical evaluation (age)	Epilepsy (onset)	Neurological examination	Oromotor weakness	Cognitive level	Other clinical features
13	LR09-216	M	2·5 yrs	39 (+2·5 SD)	57 (+4 SD) at 4 yrs	BPP grade 3	Mild ventriculomegaly, CBTE (1–5 mm), thin CC	Early developmental delays (8–10 mo)	None	Hypotonia	Expressive speech delay, difficulties chewing and swallowing	Mild ID, walked with support 18 mo, 3–4 words at 18 mo, poor coordination	Skin hyperextensibility
14	LP99-083	F	16 yrs	1st available OFC 49·6 cm at 10m (+3·6 SD)	56 cm (+3·8 SD) at 5 yrs 7 mo	BPP grade 3–4	Thick CC, mild CBTE (3 mm)	Developmental delays, macrocephaly (10 mo)	Rare generalized tonic-clonic seizures (12 yrs), off AED	Spastic quadriparesis	Profound oral dysphagia, minimal to no oral motor control. Non-verbal	Severe ID, at 14 yrs, walks short distances on knees, uses 3–4 signs, points and uses iPad pictures to indicate needs.	A few episodes of mild ketotic hypoglycemia at 5–6 yrs, subsequently resolved
15	LR11-322	F	2·5 yrs	ND	50 (+2 SD) at 22 mo	BPP grade 3	Thick CC	No details available	Epilepsy, no details available	No details available	No details available	Significant ID. Crawls and babbles at 22 mo; not walking	Icthyosis, consanguineous parents
16	LR13-409	F	4 yrs	ND (born in El Salvador)	54 (+2·5) at 3·95 yrs	BPP grade 3	Moderate ventriculomegaly	Global developmental delay, macrocephaly, static encephalopathy, diffuse hypotonia (4 mo)	Complex febrile seizures (6 mo), myoclonic jerks	Hypotonia	Dysphagia, G-tube dependent, poor vocalizations	Severe ID, non-ambulatory	G-tube dependent, temperature dysregulation
17	LR13-302	F	3 yrs	40 (+5 SD)	54 (+3) at 3 yrs	BPP grade 3	Mildly thick CC, prominent PV spaces	Macrocephaly (birth)	None	No details available	Increased drooling, no dysphagia	Developmental regression at 18 mo (had 30 words, all lost), loss of social skills, non-verbal	Severely autistic, small cutaneous capillary malformation
18	1734P	M	14 yrs	38 (+2 SD)	59 (+2–3) at 14 yrs	BPP grade 3	Ventriculomegaly (L>R)	Epilepsy (18 mo)	Rare focal seizures with unresponsiveness (18 mo)	Normal	Dysarthria	Within average (FSIQ: 78, PIQ: 78, VIQ: 97)[1]	None
19	1317N	F	22 yrs	40 (+3 SD)	60 (+3 SD) at 22 yrs	BPP grade 3	Ventriculomegaly	Language delay (3.5 yrs)	Frequent focal seizures with unresponsiveness (4.2 yrs)	Sialorrhea	Dysarthria, increased drooling	Mild disability (FSIQ: 41, VCI: 55, POI: 52, WMI: 53, PSI: 58)[2]; mild impairment in adaptive skills	None
20	LR11-278	M	4 yrs	40·5 (+5 SD)	60.2 cm (+7–8) at 4 yrs 4 mo	BPP grade 1–2	Moderate ventriculomegaly, thin CC, prominent PV spaces, CSPV	Megalencephaly and PMG (in utero)	Focal seizures with unresponsiveness (15 mo)	Hypotonia	Dysphagia, dysarthria, increased drooling	Mild ID, normal gross and fine motor skills	LGA

*Additional relevant clinical information:

LR08-305[a]: this child is part of a large sibship of African-American ancestry that consists of 11 children, including five affected ones (Supplementary Figure 2, family 3). Dysmorphic features seen in the affected child include heavy eyebrows, synophrys, deep set eyes, long eyelashes, full lips, broad looking thumbs, clinodactyly, large great toes. This child's mother, also mutation-positive, is known to have macrocephaly, hydrocephalus, epilepsy and schizoaffective disorder, with limited additional medical data. Therefore, this mother was not considered independently in this manuscript.

Abbreviations:

[1]WISC-R;

[2]WAIS-IV.

Abbreviations: AED = anti-epileptic drugs; CBTE = cerebellar tonsillar ectopia; CC = corpus callosum; CSPV = cavum septum pellucidum et vergae; DB = database number; F = female; FSIQ = full scale intellectual quotient; GERD = gastro-esophageal reflux; GI = gastrointestinal; ID = intellectual disability; IQ = intelligence quotient; LD = learning disability; LGA = large for gestational age; M = male; mo = months; MC = myoclonic; OFC = occipito-frontal circumference; PIQ = performance intellectual quotient; PMG = polymicrogyria; POI = perceptual organization index; PSI = processing speed index; PV = perivascular; SD = standard deviation; US = ultrasound; VCI = verbal comprehension index; VIQ = verbal intellectual quotient; VSD = ventricular septal defect; WM = white matter; WMI = working memory index; Wt = weight; yrs = years, BSID = Bayley scale of infant development.

Table 3

Mutations, levels of mosaicism and methods of detection of *PIK3R2* mutation-positive patients (N=20) [*PIK3R2*, NM_005027.2]

N	DB#	cDNA change	Amino acid change	Germline or mosaic	Tissue tested	Alternate allele fractions (AAF)[a]	Testing method	Inheritance
Constitutional *PIK3R2* mutations								
1	LR11-321	c.1117G>A	p.Gly373Arg	Germline	Blood	115/262 (43·9%)	smMIPs, Sanger	*De novo*
2	LR12-099	c.1117G>A	p.Gly373Arg	Germline	Blood	171/388 (44·1%)	smMIPs, Sanger	*De novo*
3	LR12-415	c.1117G>A	p.Gly373Arg	Germline	Blood Saliva	146/321 (45·4%) 132/316 (41·7%)	smMIPs, Sanger	*De novo*
4	LR12-303	LRc.1117G>A	p.Gly373Arg	Possibly germline	Saliva	125/251 (49·8%)	smMIPs, Sanger	*De novo*
5	LR13-242	c.1117G>A	p.Gly373Arg	Germline	Blood	Heterozygous[b]	WES	*De novo*
6	LR13-298	c.1117G>A	p.Gly373Arg	Germline	Blood Saliva	N/A (50·0%) N/A (50·0%)	Sanger	*De novo*
7 P	LR08-305 LR08-305m	c.1117G>A c.1117G>A	p.Gly373Arg p.Gly373Arg	Germline Germline	Blood Blood	23/48 (47·9%) 33/80 (41·3%)	smMIPs, Sanger smMIPs, Sanger	Maternal N/A
8	LR12-319	c.1117G>A	p.Gly373Arg	Possibly germline	Saliva	102/219 (46·6%)	smMIPs, Sanger	*De novo*
9	LR13-088	c.1117G>A	p.Gly373Arg	Germline	Saliva Blood	33/71 (46·4%) N/A (50·0%)	smMIPs Sanger	*De novo*
10	LR13-157a1[c]	c.1117G>A	p.Gly373Arg	Germline	Blood	N/A (50·0%)	Sanger	Presumed parental germline mosaicism
11	LR13-157a2[c]	c.1117G>A	p.Gly373Arg	Germline	Blood	N/A (50·0%)	Sanger	
P	LR13-137f	c.1117G>A	p.Gly373Arg	–	Blood	0/494 (0·00%)	smMIPs	
P	LR13-157m	c.1117G>A	p.Gly373Arg	–	Blood	0/263 (0·00%)	smMIPs	
12	LR08-308	c.1126A>G	p.Lys376Glu[d]	Germline	Blood	111/197 (56·3%)	smMIPs, Sanger	*De novo*
Mosaic *PIK3R2* mutations								
13	LR09-216	c.1117G>A	p.Gly373Arg	Mosaic	Blood	10/377 (2·6%)	smMIPs, Sanger	*De novo*
14	LP99-083	c.1117G>A	p.Gly373Arg	Mosaic	Blood	41/778 (5·20%)	Agilent SureSelect	N/A
15	LR11-322	c.1117G>A	p.Gly373Arg	Mosaic	Blood	36/493 (7·3%)	smMIPs	*De novo*
16	LR13-409	c.1117G>A	p.Gly373Arg	Mosaic	Blood	37/216 (17·1%)	smMIPs	N/A
17	LR13-302	c.1117G>A	p.Gly373Arg	Mosaic	Saliva Blood	17/53 (32·0%) Undetectable	smMIPs Sanger	*De novo*
18	1734P	c.1117G>A	p.Gly373Arg	Mosaic	Blood Blood Saliva	10/86 (11·6%) 565/5453 (10·4%) 2030/6889 (29·4%)	WES Amplicon sequencing Amplicon sequencing	*De novo*

N	DB#	cDNA change	Amino acid change	Germline or mosaic	Tissue tested	Alternate allele fractions (AAF)[a]	Testing method	Inheritance
19	1317N	c.1117G>A	p.Gly373Arg	Mosaic	Blood Blood Saliva	20/132 (15%) 861/6449 (13·3%) 275/634 (43·4%)	WES Amplicon sequencing Amplicon sequencing	*De novo*
20	LR11-278	c.1117G>A	p.Gly373Arg	Mosaic	Saliva Skin Blood Lipoma	39/106 (36·7%) 144/561 (25·6%) 117/1052 (11·1%) 1/7 (14·2%)	smMIPs, Sanger	*De novo*

The genomic coordinates for these mutations are: chr19:g.18273784G>A (p.Gly373Arg), and chr19:g.18273793A>G (p.Lys376Glu)

[a] Alternate allele fractions (AAF) are based on the number of alternate or non-reference/total alleles (%).

[b] This patient underwent trio-based clinical whole exome sequencing. 99·5% of *PIK3R2* was covered at a minimum of 10X. Overall mean depth of coverage was 759X, with a quality threshold of 99·8%.

[c] Poor DNA quality. Therefore, next generation sequencing was not performed. No other tissue sources were available to analyze on this family.

[d] This mutation is not present in any of the public databases (dbSNP138, 1000 genomes, EVS, ExAC Server). It affects an evolutionarily conserved amino acid residue and is predicted to be damaging using multiple in-silico prediction programs (SIFT, Polyphen-2, MutationTaster).

Abbreviations: AAF = alternate allele fraction; f = father; m = mother; N/A = not available; NGS = next generation sequencing; P = parents; smMIPs = single molecule molecular inversion probes.