Characterization of mutations of the phosphoinositide-3-kinase regulatory subunit, \textit{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
PEAR, http://www.exelixis-lab.org/web/software/pear
Primer3, http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/

Contributors

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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 \textit{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 \textit{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, \textit{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 \textit{PIK3R2} mutations in 17 additional children. In parallel, one patient was found to have the recurrent \textit{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 \textit{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**

\textit{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).\textsuperscript{1} Bilateral perisylvian polymicrogyria (BPP) is the most common subtype of polymicrogyria,\textsuperscript{2} and was first reported as a distinct anatomoclinical syndrome in 1993.\textsuperscript{3} Many heterogeneous non-genetic and genetic etiologies have been proposed for polymicrogyria, in general, and BPP in particular.\textsuperscript{4} Extrinsic non-genetic etiologies include vascular or hypoxemic insults (e.g. twin-twin transfusion syndrome), and congenital cytomegalovirus infection.\textsuperscript{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 – \textit{RTTN} – has been associated with isolated BPP in two unrelated families.\textsuperscript{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 \textit{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. 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 ≤5X 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—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. In line with previous studies we identified approximately 7000 variants in each exomed individual and an average of 245 variations per subject after filtering. 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, 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

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PIK3R2 variant in our cohort using Fisher’s exact test (p-value < 2.2 × 10<sup>−16</sup>) (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. 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). 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. 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. Among the genetic causes, BPP has most often been reported in individuals with copy number variants, especially 1p36.3 and 22q11.2 deletion syndromes. However, genetic heterogeneity has been proposed based on reports of large families with possible autosomal dominant or X-linked inheritance with incomplete penetrance. 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). However, only for the TUBA1A, TUBB2B and OCLN genes has polymicrogyria been neuropathologically demonstrated. 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 architectural 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). PIK3R2 mutations specifically cause the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH), a relatively rare developmental brain disorder characterized by megalencephaly, polymicrogyria, ventriculomegaly often leading to hydrocephalus, and postaxial polydactyly. To date, mutations of PIK3R2 have been reported in 15 individuals with this syndrome. Mutations in two additional core pathway genes – AKT3 and CCND2 – have recently been associated with MPPH as well. 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.

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. 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. 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). Our findings show that mosaic mutations of PIK3R2 cause a regional brain malformation, similar to our experience with PIK3CA. 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. The closure of the frontal and temporal opercula over the insula is among the most complex morphological changes occurring in the postembryonic cerebral hemispheres. 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. 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.

Acknowledgments

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We thank the patients, their families and referring physicians for their contribution to our ongoing work on these disorders.

References


Whole exome sequencing (WES) of 8 child-parent trios with BPP

For details regarding WES metrics, see material and methods

Sequence read mapping and variant calling

Variant analysis and validation

Summary of variant analysis and validation:
- Prioritization of variations in candidate genes: search for rare de novo variations occurring in the same gene and in more than one patient
- Exclusion of:
  - Intergenic and intronic variants
  - Known SNVs in public, in-house, EVS, and ExAC databases
- Sanger validation and analysis of prioritized variations in patients and parents

Identification of 2 patients with the same recurrent de novo variation in PIK3R2 (c.1117G>A, p.Gly373Arg)

Deep targeted sequencing of DNA from blood and saliva: validation and quantification of somatic mosaicism

Targeted sequencing of a cohort of 118 children with polymicrogyria divided into two groups (by testing method):
- 38 children with polymicrogyria with and without fulfilling the diagnostic criteria for the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) tested by smMIPs (entire PIK3R2 gene tested)
- 80 children with polymicrogyria broadly tested by Amplicon sequencing (the p.Gly373Arg mutation tested)

Summary of genetic findings validating implication of PIK3R2 in BPP (N = 20)
- Identification of a recurrent PIK3R2 mutation (c.1117G>A, p.Gly373Arg) in 19 patients and a de novo missense mutation (c.1126A>G, p.Lys376Glu) in 1 patient with BPP
  - 13 patients had BPP with diagnostic criteria fulfilling MPPH
  - 7 patients had BPP with normal OFC
- PIK3R2 mutations were mosaic (somatic) in 8 patients and constitutional in 12 patients
- Among the constitutional mutations, mutations were de novo, except for two families:
  - One family with parent-child transmission
  - One family with presumed parental germline mosaicism

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)

<table>
<thead>
<tr>
<th>Cohort/Feature</th>
<th>Mutation-positive patients</th>
<th>Mutation-negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constitutional PIK3R2</td>
<td>Mosaic PIK3R2 mutations</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td>8 F, 12 Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/12 African American</td>
</tr>
<tr>
<td>OFC Measurements</td>
<td></td>
<td>3-8/12-12</td>
</tr>
<tr>
<td>Mean OFC (in SD) at birth for females/males</td>
<td>3-8/4-8</td>
<td>4-3-1</td>
</tr>
<tr>
<td>Mean OFC (in SD) at last assessment for females/males</td>
<td>3-75/3-75</td>
<td>2-84-7</td>
</tr>
<tr>
<td>Age range of last assessment</td>
<td>14mo–8yrs/3mo–18yrs</td>
<td>7mo–22yrs/4mo–14yrs</td>
</tr>
<tr>
<td>Megalencephaly (OFC &gt;2 SD)</td>
<td>9/12 (75%)</td>
<td>7/8 (88%)</td>
</tr>
<tr>
<td>Brain Imaging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymicrogyria (BPP) Grade 1–2</td>
<td>10/12 (83%)</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td>Polymicrogyria (BPP) Grade 3–4</td>
<td>2/12 (17%)</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>Ventriculomegaly</td>
<td>12/12 (100%)</td>
<td>5/8 (63%)</td>
</tr>
<tr>
<td>Hydrocephalus (s/p shunting)</td>
<td>1/12 (8%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>Thick corpus callosum</td>
<td>5/12 (42%)</td>
<td>3/8 (38%)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>7/12 (58%)</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td>Mean age of seizure onset</td>
<td>11 mo</td>
<td>3-89 yrs</td>
</tr>
<tr>
<td>SD</td>
<td>8-58 mo</td>
<td>4-74 yrs</td>
</tr>
<tr>
<td>Oromotor weakness</td>
<td>9/12 (75%)</td>
<td>7/8 (88%)</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>N</th>
<th>DB#</th>
<th>Sx</th>
<th>Age</th>
<th>OFC (cm) at birth</th>
<th>OFC concordant measurement</th>
<th>Polyomicrogyria</th>
<th>Additional brain abnormalities</th>
<th>Reason of first medical evaluation (age)</th>
<th>Syphony (age)</th>
<th>Neurological condition</th>
<th>Osteosclerotic lesions</th>
<th>Cognitive level</th>
<th>Other clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LR11-351</td>
<td>F</td>
<td>2.5 yrs</td>
<td>44 (+7.5 SD)</td>
<td>BPP grade 1-2</td>
<td>Mild ventriculomegaly, dysplasia</td>
<td></td>
<td></td>
<td>Moderate ventriculomegaly, dysplasia</td>
<td></td>
<td>No details available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LR12-069</td>
<td>F</td>
<td>3 yrs</td>
<td>43 (+7)</td>
<td>BPP grade 1-2</td>
<td>Moderate ventriculomegaly, thick/CC thin WM</td>
<td></td>
<td></td>
<td>Preoperative macrocephaly (3 months)</td>
<td></td>
<td>No details available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>LR12-341</td>
<td>M</td>
<td>19 yrs</td>
<td>40 (+5)</td>
<td>BPP grade 1-2</td>
<td>Moderate ventriculomegaly, thick CC</td>
<td></td>
<td></td>
<td>Microcephaly and hypotonia (unusual)</td>
<td></td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>LR12-303</td>
<td>F</td>
<td>14 yrs</td>
<td>37.7 (±3.5 yrs)</td>
<td>BPP grade 3-4</td>
<td>Present PIK3R2 type BPP</td>
<td></td>
<td></td>
<td>Febrile seizure, PMG, MRI (before birth)</td>
<td></td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>LR11-242</td>
<td>M</td>
<td>5 yrs</td>
<td>41.9 (+5.5)</td>
<td>BPP grade 1-2</td>
<td>Moderate ventriculomegaly, thin/CC, prominent PV spaces, CSPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Speech delay</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>LR13-298</td>
<td>F</td>
<td>8 yrs</td>
<td>55 (+4-5 yrs)</td>
<td>BPP grade 1-2</td>
<td>Hydrocephalus, ventriculomegaly (10 months, CHTE 0-5 mm, abnormal CC, thin WM)</td>
<td></td>
<td></td>
<td>Ventriculomegaly, ex-vacuo (5 months, GA 34 weeks)</td>
<td></td>
<td>Axial hypotonia, appendicular hypotonia</td>
<td>Dysphagia, dysarthria, progressive hearing loss</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>LR03-305</td>
<td>M</td>
<td>6 yrs</td>
<td>No details available</td>
<td>BPP grade 1-2</td>
<td>Mild ventriculomegaly, split/CC, thin/CC (1-5 mm, mildly thick CC)</td>
<td></td>
<td></td>
<td>Febrile seizures (5 weeks), macrocephaly (7 months)</td>
<td></td>
<td>Normal</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>LR12-319</td>
<td>F</td>
<td>4 yrs</td>
<td>39 (+3)</td>
<td>BPP grade 1-2</td>
<td>Severe microcephaly, thin/CC, marked PV</td>
<td></td>
<td></td>
<td>Focal seizures with unresponsiveness (1 and 3 weeks)</td>
<td></td>
<td>No</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>LR12-305</td>
<td>F</td>
<td>2 yrs</td>
<td>No details available</td>
<td>BPP grade 1-2</td>
<td>Moderate ventriculomegaly, CHTE 0-5 mm, mildly thick CC</td>
<td></td>
<td></td>
<td>Developmental delay (6 months)</td>
<td></td>
<td>Hemiparesis</td>
<td>Expansive speech delay, increased hearing</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>LR13-157A</td>
<td>F</td>
<td>8.5 yrs</td>
<td>38 (+2)</td>
<td>BPP grade 1-2</td>
<td>Ventriculomegaly</td>
<td></td>
<td></td>
<td>Scars forming at 9 months</td>
<td></td>
<td>Normal</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>LR13-157B</td>
<td>M</td>
<td>4 yrs</td>
<td>40.5 (±4.5)</td>
<td>BPP grade 1-2</td>
<td>Mild ventriculomegaly</td>
<td></td>
<td></td>
<td>Microcephaly, craniosynostosis, facial dysmorphism</td>
<td></td>
<td>No</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

*Patients with the constitutional c.1117G>A, p.Gly373Arg PIK3R2 mutation*
<table>
<thead>
<tr>
<th>N</th>
<th>DB#</th>
<th>Sex</th>
<th>Age</th>
<th>OFC (cm) at birth</th>
<th>OFC (cm) at last assessment</th>
<th>Polymicrogyria</th>
<th>Additional brain abnormalities</th>
<th>Reason for first medical evaluation (age)</th>
<th>Epilepsy (month)</th>
<th>Neurological examination</th>
<th>Oromotor evaluation</th>
<th>Cognitive level</th>
<th>Other clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>LR09-216</td>
<td>M</td>
<td>25 yrs</td>
<td>59 (+5 SD) at birth</td>
<td>37 (+4 SD at 4 yrs)</td>
<td>RPP grade 3</td>
<td>Mildly thick CC, prominent PV spaces</td>
<td>Early developmental delays (6–11 mos)</td>
<td>None</td>
<td>Hypotonia</td>
<td>Expansive spasticity, difficulty walking and standing</td>
<td>MI, ID, walked with support 18 mos, 5 yrs with walker (18 mos)</td>
<td>Malignant hyperthermia</td>
</tr>
<tr>
<td>14</td>
<td>LP99-083</td>
<td>F</td>
<td>16 yrs</td>
<td>56 (+3 SD at 6mos)</td>
<td>56 (+5 SD at 23 mos)</td>
<td>RPP grade 3-4</td>
<td>Thick CC, mild CBTE (3 mos)</td>
<td>Developmental delays, microcephaly (10 mos)</td>
<td>Rare generalized tonic-clonic seizures (12 yrs)</td>
<td>None</td>
<td>Hypotonia</td>
<td>Profound hypotonia, minimal to no motor control</td>
<td>Severe ID, 3–14 yrs, severe short stature, moderate to severe ventriculomegaly, poor feeding, agenesis of corpus callosum</td>
</tr>
<tr>
<td>15</td>
<td>LR11-322</td>
<td>F</td>
<td>25 yrs</td>
<td>50 (+2 SD at 22 mos)</td>
<td>57 (+4 SD) at last assessment</td>
<td>None</td>
<td>No details available</td>
<td>Epilepsy (at 13 mos)</td>
<td>None</td>
<td>No details available</td>
<td>No details available</td>
<td>Significant ID, Gross and fine handwriting at 22 mos, non-verbal intelligence, incontinence</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>LR13-409</td>
<td>F</td>
<td>4 yrs</td>
<td>ND (born in El Salvador)</td>
<td>54 (+5 SD at 5 mos)</td>
<td>RPP grade 3</td>
<td>Moderate ventriculomegaly</td>
<td>Global developmental delay, microcephaly, microphthalmia, dysmorphic features</td>
<td>Complex block seizures (4 mos), myoclonic jerks</td>
<td>None</td>
<td>Hypotonia</td>
<td>Dyshyper trophy, Grade I dentition, poor vocalizations</td>
<td>Severe ID, no ambulatory mobility</td>
</tr>
<tr>
<td>17</td>
<td>LR13-502</td>
<td>F</td>
<td>5 yrs</td>
<td>40 (+5 SD)</td>
<td>38 (+3 SD at 3 yrs)</td>
<td>RPP grade 3</td>
<td>Mildly thick CC, prominent PV spaces</td>
<td>Macrocephaly (birth)</td>
<td>None</td>
<td>No details available</td>
<td>Speech delay, no dysphagia</td>
<td>Developmental regression at 18 mos (first steps, all four permanent teeth), loss of social skills, severe ID, severe ID, no ambulatory mobility</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>17MP</td>
<td>M</td>
<td>14 yrs</td>
<td>58 (+2–3 SD at 14 mos)</td>
<td>38 (+3 SD) at 14 mos</td>
<td>RPP grade 3</td>
<td>Ventriculomegaly (18 mos)</td>
<td>Epilepsy (10 mos)</td>
<td>Rare focal seizures with unresponsiveness (10 mos)</td>
<td>Normal</td>
<td>Dysarthria</td>
<td>Metaphyseal dysplasia, restrictive growth failure</td>
<td>Severe arthrogryposis, severe kyphoscoliosis</td>
</tr>
<tr>
<td>19</td>
<td>1317N</td>
<td>F</td>
<td>22 yrs</td>
<td>40 (+3 SD at 22 mos)</td>
<td>40 (+3 SD at 22 mos)</td>
<td>RPP grade 3</td>
<td>Ventriculomegaly</td>
<td>Language delay (3.5 yrs)</td>
<td>Frequent focal seizures with unresponsiveness (4.2 yrs)</td>
<td>Scoliosis</td>
<td>Dysarthria, toe walking</td>
<td>Mild dysarthria (18 mos), VQ 55, PIQ 52, WMI 37, PIQ 57, IDL 422, incontinence, autistic traits</td>
<td>None</td>
</tr>
<tr>
<td>20</td>
<td>LR13-275</td>
<td>M</td>
<td>4 yrs</td>
<td>60 (+5 SD at 17 mos)</td>
<td>40 (+1 SD at 6 yrs)</td>
<td>RPP grade 1-2</td>
<td>Moderate ventriculomegaly (16 mos), prominent PV spaces, CSPV</td>
<td>Focal seizures with unresponsiveness (13 mos)</td>
<td>None</td>
<td>Hypotonia</td>
<td>Dyshypermotria, toe walking</td>
<td>Mild ID, normal gross and fine motor skills</td>
<td>LGA</td>
</tr>
</tbody>
</table>

Additional relevant clinical information:

LR08-305: 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 schizophrenia 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; FSQ = full scale intellectual quotient; GEDD = gastro-esophageal reflux disease; GI = gastrointestinal; ID = intellectual disability; IQ = intelligence quotient; LD = learning disability; LGA = large for gestational age; M = male; mo = months; MC = myoclonus; OFC = occipito-frontal circumference; PIQ = performance intellectual quotient; PMG = polymicrogyria; POI = perceptual organization index; PSQ = 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]

<table>
<thead>
<tr>
<th>N</th>
<th>DB#</th>
<th>cDNA change</th>
<th>Amino acid change</th>
<th>Germline or mosaic</th>
<th>Tissue tested</th>
<th>Alternate allele fractions (AAF)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Testing method</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Constitutional PIK3R2 mutations</td>
</tr>
<tr>
<td>1</td>
<td>LR11-321</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>115/262 (43.9%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td>2</td>
<td>LR12-099</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>171/388 (44.1%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td>3</td>
<td>LR12-415</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>146/321 (45.4%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Saliva</td>
<td>152/316 (47.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>LR12-303</td>
<td>LRc.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Possibly germline</td>
<td>Saliva</td>
<td>125/251 (49.8%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td>5</td>
<td>LR13-242</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>Heterozygous&lt;sup&gt;b&lt;/sup&gt;</td>
<td>WES</td>
<td>De novo</td>
</tr>
<tr>
<td>6</td>
<td>LR13-298</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>N/A (50-0%)</td>
<td>Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Saliva</td>
<td>N/A (50-0%)</td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td>LR08-305</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>23/48 (47.9%)</td>
<td>smMIPs, Sanger</td>
<td>Maternal</td>
</tr>
<tr>
<td></td>
<td>LR08-305m</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>33/80 (41.3%)</td>
<td>smMIPs, Sanger</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>LR12-319</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Possibly germline</td>
<td>Saliva</td>
<td>102/219 (46.6%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td>9</td>
<td>LR13-088</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Saliva</td>
<td>33/71 (46.4%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood</td>
<td>N/A (50-0%)</td>
<td></td>
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</tr>
<tr>
<td>10</td>
<td>LR13-157a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>N/A (50-0%)</td>
<td>Sanger</td>
<td>Presumed parental germline mosaicism</td>
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<tr>
<td>11</td>
<td>LR13-157a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Germline</td>
<td>Blood</td>
<td>N/A (50-0%)</td>
<td>Sanger</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>LR13-137f</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>–</td>
<td>Blood</td>
<td>0/494 (0-0%)</td>
<td>smMIPs</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>LR13-157m</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>–</td>
<td>Blood</td>
<td>0/263 (0-0%)</td>
<td>smMIPs</td>
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<tr>
<td>12</td>
<td>LR08-308</td>
<td>c.1126A&gt;G</td>
<td>p.Lys376Glu&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Germline</td>
<td>Blood</td>
<td>111/197 (56-3%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
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<td>Mosaic PIK3R2 mutations</td>
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<td>13</td>
<td>LR09-216</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>10/377 (2.6%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
</tr>
<tr>
<td>14</td>
<td>LP99-083</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>41/778 (52.0%)</td>
<td>Agilent SureSelect</td>
<td>N/A</td>
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<tr>
<td>15</td>
<td>LR11-322</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>36/493 (7.3%)</td>
<td>smMIPs</td>
<td>De novo</td>
</tr>
<tr>
<td>16</td>
<td>LR13-409</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>37/216 (17.1%)</td>
<td>smMIPs</td>
<td>N/A</td>
</tr>
<tr>
<td>17</td>
<td>LR13-302</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>17/31 (55.0%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
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<td>Saliva</td>
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<td>18</td>
<td>1734P</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>10/86 (11.6%)</td>
<td>WES</td>
<td>De novo</td>
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<td>Saliva</td>
<td>565/5433 (10-4%)</td>
<td>Amplicon sequencing</td>
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<td>2030/6889 (29-4%)</td>
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<td>N</td>
<td>DB#</td>
<td>cDNA change</td>
<td>Amino acid change</td>
<td>Germline or mosaic</td>
<td>Tissue tested</td>
<td>Alternate allele fractions (AAF)</td>
<td>Testing method</td>
<td>Inheritance</td>
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<tr>
<td>19</td>
<td>1317N</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>20/132 (15%)</td>
<td>WES, Amplicon sequencing</td>
<td>De novo</td>
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<td>Blood</td>
<td>861/8449 (13·3%)</td>
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<td></td>
<td>Saliva</td>
<td>275/634 (43·4%)</td>
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</tr>
<tr>
<td>20</td>
<td>LR11-278</td>
<td>c.1117G&gt;A</td>
<td>p.Gly373Arg</td>
<td>Mosaic</td>
<td>Blood</td>
<td>39/106 (36·7%)</td>
<td>smMIPs, Sanger</td>
<td>De novo</td>
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<td></td>
<td>Skin</td>
<td>144/561 (25·6%)</td>
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<td></td>
<td>Blood</td>
<td>117/1052 (11·1%)</td>
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<td>Lipoma</td>
<td>1/7 (14·2%)</td>
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</table>

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.