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

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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. The causes of BPP are heterogeneous, but only a few genetic causes have been reported. The aim of this study was to identify additional genetic causes of BPP and characterise their frequency in this population.

Methods Children (aged ≤18 years) with polymicrogyria were enrolled into our research programme from July, 1980, to October, 2015, at two centres (Florence, Italy, and Seattle, WA, USA). We obtained samples (blood and saliva) throughout this period at both centres and did whole-exome sequencing on DNA from eight trios (two parents and one affected child) with BPP in 2014. After the identification of mosaic *PIK3R2* mutations in two of these eight children, we performed targeted screening of *PIK3R2* by two methods in a cohort of 118 children with BPP. First, we performed targeted sequencing of the entire *PIK3R2* gene by single molecule molecular inversion probes (smMIPs) on 38 patients with BPP with normal to large head size. Second, we did amplicon sequencing of the recurrent *PIK3R2* mutation (Gly373Arg) in 80 children with various types of polymicrogyria including BPP. One additional patient had clinical whole-exome sequencing done independently, and was included in this study because of the phenotypic similarity to our cohort.

Findings We identified a mosaic mutation (Gly373Arg) in a regulatory subunit of the PI3K-AKT-mTOR pathway, *PIK3R2*, in two children with BPP. Of the 38 patients with BPP and normal to 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 had the recurrent *PIK3R2* mutation identified by clinical whole-exome sequencing. Seven of these 20 patients had BPP alone, and 13 had BPP in association with features of the megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome. 19 patients had the same mutation (Gly373Arg), and one had a nearby missense mutation (Lys376Glu). Mutations were constitutional in 12 patients and mosaic in eight patients. In patients with mosaic mutations, we noted substantial variation in alternate (mutant) allele levels, ranging from ten (3%) of 377 reads to 39 (37%) of 106 reads, equivalent to 5–73% of cells analysed. Levels of mosaicism varied from undetectable to 37 (17%) of 216 reads in blood-derived DNA compared with 2030 (29%) of 6889 reads to 275 (43%) of 634 reads in saliva-derived DNA.

Interpretation Constitutional and mosaic mutations in the *PIK3R2* gene are associated with developmental brain disorders ranging from BPP with a normal head size to the MPPH syndrome. The phenotypic variability and low-level mosaicism, which challenge conventional molecular methods, have important implications for genetic testing and counselling.

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Introduction

Polymicrogyria is a cortical malformation characterised by excessive gyration and disordered lamination, and is one of the most common malformations of cortical development.[1] Bilateral perisylvian polymicrogyria (BPP) is the most common subtype of polymicrogyria,[2] and was first reported as a distinct anatomical and clinical syndrome in 1993.[3] Many heterogeneous non-genetic and genetic causes have been proposed for polymicrogyria in general, and BPP in particular.[4] Extrinsic non-genetic causes include vascular or hypoxaemic insults (eg, twin-to-twin transfusion syndrome) and congenital cytomegalovirus infection.[1] Genetic causes are collectively rare for BPP and typically occur in clinically recognisable syndromic forms, the most common of which are 1p36.3 and 22q11.2 deletion syndromes. Until now, only one

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Research in context

Evidence before this study

Polymicrogyria is a common malformation of cortical development, with an estimated prevalence of 16% in large series. However, discovery of underlying causative genes has lagged behind studies of other cortical malformations such as lissencephaly, most likely because of causative heterogeneity. Thus, discovery of new genes for polymicrogyria is likely to have a major effect on health in paediatric neurology. We searched PubMed on April 2, 2015, with the terms "(polymicrogyria OR bilateral perisylvian polymicrogyria)", and found no evidence of new genetic associations with polymicrogyria in general, and bilateral perisylvian polymicrogyria (BPP) in particular, since the identification of *RTTN* in 2012. We further searched PubMed for the term "(*PIK3R2* AND polymicrogyria)" and found no evidence of this gene being associated with isolated polymicrogyria, but only with megalencephaly-associated polymicrogyria, as we previously reported. Finally, we searched the Developmental Brain Disorders Database on April 2, 2015, with the term "(polymicrogyria)", and found no evidence of new genes associated with polymicrogyria.

Added value of this study

From our systematic review, we concluded that although mutations of *PIK3R2* are associated with megalencephaly, polymicrogyria, and hydrocephalus, no new discoveries linking mutations in this gene to isolated polymicrogyria have been reported. During the past 25 years (combining patients identified by the groups of Renzo Guerrini [Florence, Italy] and William Dobyns [Seattle, WA, USA]), we have recruited and obtained samples from more than 500 patients with polymicrogyria including more than 100 with BPP, and assessed their clinical and neuroimaging features. For whole-exome sequencing, we selected eight child–parent trios with BPP, finding *PIK3R2* mutations in two of eight patients. We then screened 118 patients for the mutations in *PIK3R2*, including 38 patients with BPP and normal to large head size (who were screened for mutations across the entire gene) and 80 patients with polymicrogyria with variable head sizes (who were screened for the recurrent *PIK3R2* mutation, Gly373Arg). Combined with one additional patient who underwent clinical exome sequencing, we identified 20 patients with BPP with *PIK3R2* mutations.

Implications of all the available evidence

In this study, we show that constitutional and mosaic *PIK3R2* mutations account for 19 (15%) of 126 patients with polymicrogyria, with mosaic mutations accounting for eight (6%) of the cohort; all *PIK3R2* mutations were in patients with BPP. Therefore, our data suggest that mutations of *PIK3R2* are the most common genetic cause of BPP in patients with normal head size, and the second (to *PIK3CA*) most common cause of polymicrogyria associated with large head size. Furthermore, our data substantiate the role of mosaicism as a cause of developmental brain disorders at large, including the role of parental germline mosaicism for *PIK3R2* specifically, and report on the first parentally inherited *PIK3R2* mutation.

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 the disorder.

Methods

Participants

This study was done at the Seattle Children's Research Institute, Seattle, WA, USA, and the University of Florence Meyer Children's Hospital, Florence, Italy. Between 1980 and 2015, we have assessed patients at both centres as part of our developmental brain disorders research programme. Patients included in this study were children aged 18 years or younger with polymicrogyria identified by brain imaging, with or without brain overgrowth (or megalencephaly). Patients with inadequate imaging or clinical data, or both, were excluded from this study. Written informed consent was obtained from all of the patients' legal guardians to share clinical, neuroimaging, and electroencephalographic data and provide key research samples including blood and saliva and skin, when available. Samples were obtained between July, 1980, and October, 2015, with increasing sample ascertainment in the past few years. Blood samples for whole-exome sequencing were collected from December, 2012, to October, 2013. Clinical and neuroimaging studies were reviewed by the investigators. This study was approved by the Seattle Children's Institutional Review Board and the Pediatric Review Board of the Tuscany Region.

Procedures

A comprehensive MRI investigation was done on every patient, using different imaging systems including 1·5-Tesla, 3-Tesla, or 7-Tesla scans. Minimum sequence requirement consisted of non-contrast-enhanced spin echo, inversion recovery, and gradient echo sequences performed in the axial, sagittal, and coronal planes. All patients were examined with slice thickness of 5 mm or less. The ultra-high field 7-Tesla MRI included three-dimensional (3D) T1-weighted fast-spoiled gradient echo, 3D susceptibility-weighted angiography, two-dimensional (2D) T2-weighted targeted dual-echo gradient-recalled echo, 2D T2-weighted dual spin echo, and 2D grey-white matter tissue border enhancement fast spin-echo inversion recovery.

Molecular methods

Genomic DNA was extracted from patients' tissues by standard protocols using the Puregene Blood Core Kit (Qiagen, Venlo, Netherlands) with RNase for blood, and the Oragene Saliva Kit (DNA genotek, Ottawa, ON, Canada) following the manufacturers' recommendations.

First, whole-exome sequencing was done on DNA samples from eight trios (two parents and one affected child) with BPP between January and September, 2014. Patients selected for whole-exome sequencing were those for whom an underlying genetic cause had not been identified by previous standard testing that included a chromosomal microarray, and who had no clinical or imaging findings suggestive of a non-genetic cause. Of these eight patients, two had megalencephaly (defined as occipitofrontal circumference [OFC] >2 SDs above the mean for age and sex), two had borderline small head size (OFC ≥2 SDs below the mean for age and sex), and the remaining four were normocephalic. The parents of all eight children were clinically unaffected. Mean OFC measurements and SDs for age and sex were calculated by use of the standard Nellhaus Head Circumference Charts for children from birth to 18 years.[6]

To further assess the frequency of the *PIK3R2* (phosphoinositide-3-kinase, regulatory subunit 2 [beta]) mutation, Gly373Arg, which was seen in two of our patients who had whole-exome sequencing (and was therefore deemed recurrent), we developed an allelic discrimination assay in November, 2014, to screen a cohort of 80 children with polymicrogyria broadly (without use of head size as a selection criterion). The presence of two primer–probe pairs marked with two different fluorescent dyes in the same allelic discrimination assay allowed us to assess the allelic status at the mutation site. In parallel, we screened 38 patients by use of single molecule molecular inversion probes (smMIPs) for mutations in *PIK3R2*.[7] These 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 (MPPH) syndrome had clinical trio-based whole-exome sequencing done independently.

Library preparation, exome enrichment, and whole-exome sequencing were done at the French National Centre for Genotyping (Evry, France). Libraries were prepared from 3 µg genomic DNA extracted from whole blood using an optimised SureSelect Human Exome kit (Agilent, Santa Clara, CA, USA). Captured, purified, and clonally amplified libraries targeting the exome were then sequenced on a HiSeq 2000 (Illumina, San Diego, CA, USA). Sequence reads were aligned to the human genome (hg19 assembly) using BWA software. Downstream processing was done with the Genome Analysis Toolkit, 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 of five times or lower and a Phred-scaled single nucleotide polymorphism quality of 20 or lower 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 100×. Coverage depth greater than 15× and 5× was obtained for about 97% and 99% of the target, respectively.

We analysed variants affecting coding regions and essential splice sites and excluded all variants with frequencies higher than 1% in multiple genome databases including the Single Nucleotide Polymorphism Database (dbSNP), 1000 Genomes, the National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server (EVS), the Exome Aggregation Consortium (ExAC), and a local Paris Descartes Bioinformatics platform database.

We designed a pool of 35 smMIP oligonucleotides targeting the coding sequences of *PIK3R2*. smMIPs were tiled across a total of 3340 bp of genomic sequence, including all 2202 coding nucleotides of the targeted gene. 100 ng capture reactions were performed in parallel. Massively parallel sequencing was done with the Illumina HiSeq. Variants were filtered against public databases (dbSNP, 1000 Genomes, EVS, ExAC). smMIP sequencing data were processed with MIPgen and PEAR version 0.8.1,[8] both with default options, with the exception of introducing a penalty of 80 for soft clipping during the BWA-MEM, to produce high quality single molecule consensus reads (smc-reads). smc-reads were analysed with GATK version 3.1-1 as recommended using the IndelRealigner and HaplotypeCaller tools on the targeted regions. smc-reads were processed with Freebayes using the –F0 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.

To screen for the recurrent *PIK3R2* mutation, Gly373Arg, we performed locus-specific amplification of genomic DNA followed by GS Junior sequencing (Roche, Branford, CT, USA). We designed fusion primers, containing genome-specific sequences along with distinct 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 from the corresponding author (RG). Small DNA fragments were removed with Agencourt AMPure XP (Beckman Coulter, Beverly, MA, USA) according to the manufacturer's protocol. All amplicons were quantified with the Quant-iT PicoGreen dsDNA reagent (Invitrogen Corporation, Life Technologies, Carlsbad, CA, USA), 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 manufacturers' instructions. Data analysis was done with GS Amplicon Variant Analyzer version 3.0 software.

For the **Single Nucleotide Polymorphism Database** see http://www.ncbi.nlm.nih.gov/SNP/

For the **NHLBI Exome Variant Server** see http://evs.gs.washington.edu/EVS/

For the **Exome Aggregation Consortium** see http://exac.broadinstitute.org

For **PEAR software** see http://www.exelixis-lab.org/web/software/pear

For **Freebayes** see https://github.com/ekg/freebayes

For **Primer3Plus software** see http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/

For the **Genome Analysis Toolkit** see https://www.broadinstitute.org/gatk/

For **SAMtools** see http://samtools.sourceforge.net/

For the **Picard Project** see http://broadinstitute.github.io/picard/

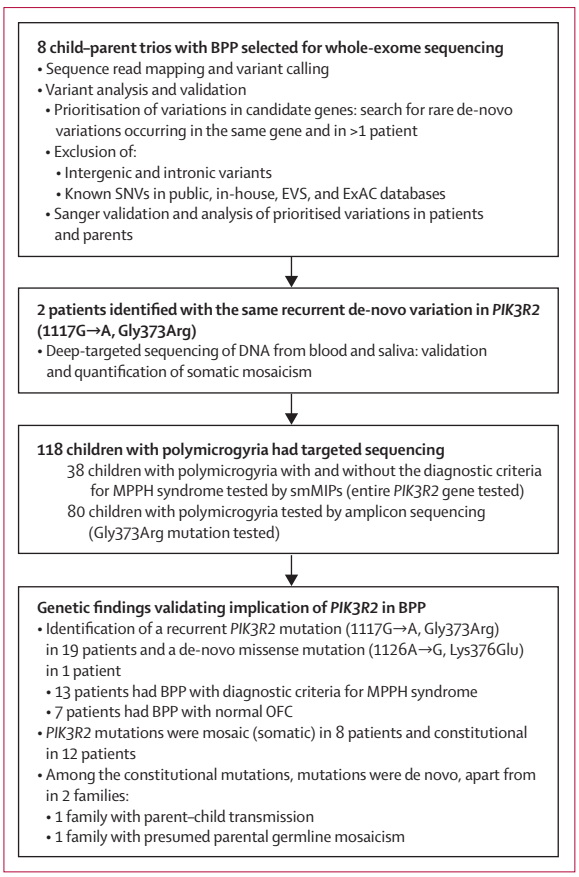

Figure 1: **Experimental workflow**
This workflow is an adaptation of the one we have previously used to search for genes associated with malformations of cortical development.[10,11] Patients selected for exome sequencing were children (aged <18 years) with polymicrogyria for whom an underlying genetic cause has not been identified by standard testing that includes a chromosomal microarray, and who have no clinical or imaging findings suggestive of a non-genetic cause. BPP=bilateral perisylvian polymicrogyria. SNV=single nucleotide variant. EVS=National Heart, Lung, and Blood Institute Exome Variant Server. ExAC=Exome Aggregation Consortium. smMIPs=single molecule molecular inversion probes. MPPH=megalencephaly-polymicrogyria-polydactyly-hydrocephalus. OFC=occipitofrontal circumference.

See Online for appendix

For **Integrated Genome Viewer** see http://www.broadinstitute.org/igv/

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

For **Primer 3** see http://bioinfo.ut.ee/primer3-0.4.0/

Statistical analysis
p values were calculated by use of Fisher's exact test and 95% CIs were calculated by the Newcombe method.[9]

Role of the funding source
The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
We tested eight child–parent trios by whole-exome sequencing (figure 1); all families had a single affected patient[10,12,13] (sporadic case) with BPP. In line with previous studies, we identified about 7000 genetic variants in each individual and a mean of 245 variations per patient after filtering. See appendix for data on quality metrics for whole-exome sequencing and the de-novo variants identified in this cohort.

Filtering of exome data and a search for variations in the same gene in unrelated patients showed the same recurrent mutation (1117G→A, Gly373Arg) in *PIK3R2* in two patients. This mutation was not present in any of the public databases. However, analysis of the reads generated by high-throughput sequencing using the Integrated Genome Viewer interface showed that this variant is present in ten (12%) of 86 reads for patient 18 and 20 (15%) of 132 reads 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 whole-exome sequencing, variation in read depth between DNA samples is caused by 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.

In view of the identification of a recurrent mosaic mutation (Gly373Arg) in *PIK3R2* in BPP, which is also the same mutation identified previously in MPPH syndrome,[14] we searched for mutations in this gene in 118 patients with polymicrogyria from a cohort of more than 500 patients accrued over the past 25 years (table 1). 38 patients who had BPP with normal or large head size (including 32 with MPPH syndrome) were tested by smMIPs and Sanger sequencing. This testing strategy identified mutations in 17 patients, 16 of whom had the same *PIK3R2* mutation (Gly373Arg) identified by whole-exome sequencing (tables 2, 3). Another patient with MPPH syndrome (patient 5), who was independently studied by clinical whole-exome sequencing, had the same *PIK3R2* mutation. One patient had a de-novo missense mutation within the same functional domain of the *PIK3R2* gene, Lys376Glu. 80 additional patients with a broad diagnosis of polymicrogyria were tested

	Mutation-positive patients		Mutation-negative patients		
	Constitutional *PIK3R2* mutations (n=12)	Mosaic *PIK3R2* mutations (n=8)	Patients with BPP; whole-exome sequencing (n=6)	Patients with polymicrogyria; amplicon sequencing (n=80*)	Patients with BPP; smMIPs (n=21)
Sex					
Male	4 (33%)	4 (50%)	2 (33%)	23 (29%)	13 (62%)
Female	8 (67%)	4 (50%)	4 (67%)	30 (38%)	8 (38%)
Unknown	0	0	0	27 (34%)	0
Ethnic origin					
White	11 (92%)	8 (100%)	6 (100%)	53 (66%)	11 (52%)
African-American	1 (8%)	0	0	0	2 (10%)
Asian	0	0	0	0	2 (10%)
Hispanic	0	0	0	0	2 (10%)
Unknown	0	0	0	27 (34%)	4 (19%)
Mean OFC (in SDs) at birth					
Male patients	4·8	3·1	0	0	3·3
Female patients	3·8	4	0	–1	1·6
Mean OFC (in SDs) at last assessment					
Male patients	5·3	4·7	–2	–0·7	3·2
Female patients	3·8	2·8	0	–1·2	4·3
Age range of last OFC assessment					
Male patients	3 months–18 years	4 months–14 years	3–7 years	1–15·5 years	13 months–7·5 years
Female patients	14 months–8·5 years	7 months–22 years	4·5–16 years	3 months–13·5 years	5·5 months–7·5 years
Megalencephaly (OFC >2 SD)	9 (75%)	7 (88%)	0	2 (4%)	19 (90%)
Brain imaging					
Polymicrogyria (BPP) grade 1–2	10 (83%)	6 (75%)	3 (50%)	24 (30%)	5 (24%)
Polymicrogyria (BPP) grade 3–4	2 (17%)	2 (25%)	3 (50%)	29 (36%)	16 (76%)
Ventriculomegaly	12 (100%)	5 (63%)	0	6 (8%)	9 (43%)
Hydrocephalus (after shunting)	1 (8%)	0	0	0	3 (14%)
Thick corpus callosum	5 (42%)	3 (38%)	0	2 (3%)	4 (19%)
Epilepsy	7 (58%)	6 (75%)	1 (17%)	37 (46%)	13 (62%)
Mean age of seizure onset (SD)	11 months (8·6)	3·89 years (4·7)	2·3 years (NA)	3·4 years (3·9)	14 months (9·9)
Oromotor weakness	9 (75%)	7 (88%)	4 (67%)	13 (25%)	10 (48%)

Data are n (%) unless otherwise stated. BPP=bilateral perisylvian polymicrogyria. NA=not available. OFC=occipitofrontal circumference. smMIPs=single molecule molecular inversion probes. *Of these 80 patients, clinical data were available for 53 patients, although all 80 patients were confirmed to have polymicrogyria by assessment of their neuroimaging; therefore, the denominator for all the clinical data is 53, and for neuroimaging data 80.

Table 1: **Clinical and neuroimaging features of all patients included in this study**

only for the recurrent *PIK3R2* mutation (1117G→A, Gly373Arg) by amplicon sequencing and all were negative for the mutation.

Because the same *PIK3R2* mutation was detected in a subset of patients with polymicrogyria among a cohort of 126 patients, we calculated the probability for the recurrent *PIK3R2* mutation occurring by chance in our cohort. Comparison of the allele frequency of the *PIK3R2* non-synonymous variant in our cohort (19/126 [15%]) with the one reported in the largest public database (ExAC; 0/33 113) showed a significant enrichment of *PIK3R2* variant in our cohort ($p<2\cdot2\times10^{-16}$; Fisher's exact test; appendix).

Across the cohort, mutations were constitutional in 12 patients (table 2) and mosaic in eight patients (table 3). In patients with mosaic mutations, we noted substantial variation in alternate (mutant) allele levels within individual samples, ranging from ten (3%) of 377 reads to 39 (37%) of 106 reads, equivalent to 5–73% of cells analysed. Levels of mosaicism varied from undetectable to 37 (17%) of 216 reads in blood-derived DNA compared with 2030 (29%) of 6889 reads to 275 (43%) of 634 reads in saliva-derived DNA (table 3). To exclude artifactual low-frequency variant detection caused by sample cross-contamination or index cross-talk, we confirmed mutations by independent captures or Sanger sequencing. Patient 12 had a different de-novo missense mutation of *PIK3R2* (1126A→G, Lys376Glu), which was not present in any of the public databases and is predicted to be pathogenic by use of in-silico analysis. This de-novo mutation also affects a highly evolutionarily conserved aminoacid residue within the sequence homology domain of PIK3R2, and is therefore predicted to be pathogenic.[15] All patients with *PIK3R2* mutations had

	Sex	Age last assessed	OFC at birth, cm (SD)	OFC at last assessment, cm (SD)	Poly-microgyria (BPP grade)	Additional brain abnormalities	Reason of first medical evaluation (age)	Epilepsy (age at onset)	Neurological examination	Oromotor weakness	Cognitive level	Other clinical features
Patients with the constitutional 1117G→A, Gly373Arg *PIK3R2* mutation												
1	F	2·5 years	44 (8)	59 (8·5) at 2·5 years	1–2	Moderate ventriculomegaly, dysmyelination	Macrocephaly (birth)	Epilepsy (NA)	NA	NA	Significant LD, global developmental delay	None
2	F	3 years	43 (7)	55·5 (4·5) at 3·5 years	1–2	Moderate ventriculomegaly, thick CC, thin white matter	Progressive macrocephaly (3 months)	No seizures	Quadriparesis	Dysphagia, speech delays (non-verbal; mimics sounds), excessive drooling	Severe ID, poor head control, wheelchair bound	Gastrointestinal malrotation, laryngomalacia
3	M	18 years	40 (4·5)	63·6 (6) at 18 years	1–2	Moderate ventriculomegaly, thick CC	Macrocephaly and hypotonia (infancy)	Rare focal seizures with unresponsiveness (2 years)	Hypotonia	Non-verbal	Severe ID (IQ <35), walked with assistance at 4 years	Divergent strabismus
4	F	14 months	39 (4)	47·7 (1) at 14 months	3–4	Prominent perivascular spaces, mild ventriculomegaly, thin CC, mild CBTE, CSPV, hippocampal dysgenesis	Eye deviation, polymicrogyria on MRI (shortly after birth)	None	Hypotonia, hypokinesia	Poor suck and swallow, poor swallow coordination, status post gastrostomy, excessive drooling, markedly delayed speech	Severe ID	Hyperopia, severe astigmatism, GORD
5	M	5 years	41·9 (5·5)	58 (5) at 5 years	3–4	Moderate ventriculomegaly, thin CC, prominent perivascular spaces, CSPV	Macrocephaly, ventriculomegaly detected on prenatal ultrasound	None	Normal	Speech delay	Mild–moderate ID, walked at 12 months, fine motor delays	Attention deficit, sensory processing issues, LGA, transient hypoglycaemia at birth
6	F	8·6 years	NA	59·5 (6) at 8·5 years	1–2	Hydrocephalus status post ventriculostomy (10 months), CBTE (1–5 mm), stretched CC, thin white matter	Ventriculomegaly on prenatal ultrasound (GA 34 weeks)	Infantile spasms evolved into myoclonic seizures (1 year), intractable	Axial hypotonia, appendicular hypertonia	Dysphagia, dysarthria, profuse drooling	Severe ID, non-ambulatory, non-verbal, no social or communication skills	Connective tissue laxity, GORD, short stature at 8 years
7*	M	6 years	NA	57 (5) at 3 years	1–2	Mild ventriculomegaly, mild CBTE (1–3 mm), mildly thick CC	Eye deviation (1 week), macrocephaly (3 months)	Focal seizures with unresponsiveness (1 year)	Hypotonia, oculomotor apraxia	Delayed speech	Moderate ID, walked at 16 months, delayed fine motor skills, 80 words at 6 years	GORD, dysmorphic facial features
8	F	4 years	39 (4)	55 (3·5) at 5 years	1–2	Severe ventriculomegaly, thin or stretched CC	Macrocephaly, multiple muscular VSDs (birth)	Focal seizures with unresponsiveness (1 month)	Hemiparesis	Dysphagia with fatigue with food	Moderate ID, walked at 2·5 years, speech delay	Multiple VSDs, esotropia, hyperopia, astigmatism, broad thumbs, sandal gap toes
9	F	2 years	NA	52 (4) at 16 months	1–2	Moderate ventriculomegaly, CBTE (1–5 mm), mildly thick CC	Developmental delays (6 months)	None	Hemiparesis	Expressive speech delay, increased drooling	Mild ID	None
10	F	8·5 years	38 (3)	55·5 (2·5) at 8·5 years	1–2	Ventriculomegaly	Seizures (commencing at 15 months)	Focal seizures with unresponsiveness (15 months)	Normal	None	Developmental delay at 2 years, behind peers, IQ not formally assessed	Cutis marmorata, weight 2 standard deviation above the mean

(Table 2 continues on next page)

	Sex	Age last assessed	OFC at birth, cm (SD)	OFC at last assessment, cm (SD)	Poly-microgyria (BPP grade)	Additional brain abnormalities	Reason of first medical evaluation (age)	Epilepsy (age at onset)	Neurological examination	Oromotor weakness	Cognitive level	Other clinical features
(Continued from previous page)												
11	M	4 years	40·5 (4·5)	47·5 (4·5) at 4 months	1–2	Mild ventriculomegaly	Macrocephaly identified on prenatal ultrasound; first seizure at 7 weeks	Focal seizures with unresponsiveness (2 months), intractable	Cortical blindness, otherwise normal neurological examination	None	Mild developmental delay. Levels at 21 months: gross motor 15 months, speech 12 months, fine motor 9 months, social 9 months	Cutis marmorata, cutaneous haemangioma
Patient with the constitutional 1126A→G, Lys376Glu *PIK3R2* mutation												
12	F	5 years	35 (0·7)	52·3 (1·5) at 5 years	1–2	Mild ventriculomegaly, mildly thick CC	Macrocephaly (3 months)	None	Hypotonia	Expressive language delays, early dysphagia	Moderate ID, walked at 4 years. BSID levels at 10 months: cognition 3 months, fine motor 2 months, social-emotional 4 months, language (receptive/ expressive 3 months, motor 3–4 months	Cutaneous capillary malformation
Patients with the mosaic 1117G→A, Gly373Arg *PIK3R2* mutation												
13	M	2·5 years	39 (3·5)	57 (4·5) at 4 years	3	Mild ventriculomegaly, CBTE (1–5 mm), thin CC	Early developmental delays (8–10 months)	None	Hypotonia	Expressive speech delay, difficulties chewing and swallowing	Mild ID, walked with support 18 months, 3–4 words at 18 months, poor coordination	Skin hyper-extensibility
14	F	16 years	†49·6 (3·5)	56 (4) at 5 years 7 months	3–4	Thick CC, mild CBTE (3 mm)	Developmental delays, macrocephaly (10 months)	Rare generalised tonic-clonic seizures (12 years), off anti-epileptic drugs	Spastic quadriparesis	Profound oral dysphagia, minimal to no oral motor control; non-verbal	Severe ID, at 14 years walks short distances on knees, uses 3–4 signs, points and uses iPad pictures to indicate needs	A few episodes of mild ketotic hypoglycaemia at 5–6 years, subsequently resolved
15	F	2·5 years	NA	50 (2) at 22 months	3	Thick CC	NA	Epilepsy (NA)	NA	NA	Significant ID, crawls and babbles at 22 months; not walking	Ichthyosis, consanguineous parents

(Table 2 continues on next page)

BPP, with or without megalencephaly. The most distinctive phenotypic characteristics of these patients were polymicrogyria, megalencephaly, ventriculomegaly, epilepsy, and oromotor weakness (table 2).

Polymicrogyria only affected the perisylvian cortex or extended beyond it with perisylvian predominance. Severity 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 included one or both poles with the sylvian fissures extended posteriorly and often oriented superiorly (grade 1).[2] The extent of involvement was bilateral, but often mildly asymmetric in most individuals.

13 (65%) of 20 individuals had megalencephaly defined as OFC more than 2 SD above the mean for age

	Sex	Age last assessed	OFC at birth, cm (SD)	OFC at last assessment, cm (SD)	Poly-microgyria (BPP grade)	Additional brain abnormalities	Reason of first medical evaluation (age)	Epilepsy (age at onset)	Neurological examination	Oromotor weakness	Cognitive level	Other clinical features
(Continued from previous page)												
16	F	4 years	NA	54 (2·5) at 3·95 years	3	Moderate ventriculomegaly	Global developmental delay, macrocephaly, static encephalopathy, diffuse hypotonia (4 months)	Complex febrile seizures (6 months), myoclonic jerks	Hypotonia	Dysphagia, gastrostomy dependent, poor vocalisations	Severe ID, non-ambulatory	Gastrostomy dependent, temperature dysregulation
17	F	3 years	40 (5)	54 (3) at 3 years	3	Mildly thick CC, prominent perivascular spaces	Macrocephaly (birth)	None	NA	Increased drooling, no dysphagia	Developmental regression at 18 months (had 30 words, all lost), loss of social skills, non-verbal	Severely autistic, small cutaneous capillary malformation
18	M	14 years	38 (2)	59 (2·5) at 14 years	3	Ventriculomegaly (asymmetric)	Epilepsy (18 months)	Rare focal seizures with unresponsiveness (18 months)	Normal	Dysarthria	Within average (FSIQ: 78, PIQ: 78, VIQ: 97)‡	None
19	F	22 years	40 (3)	60 (3) at 22 years	3	Ventriculomegaly	Language delay (3·5 years)	Frequent focal seizures with unresponsiveness (4·2 years)	Sialorrhoea	Dysarthria, increased drooling	Mild disability (FSIQ: 41, VCI: 55, POI: 52, WMI: 53, PSI: 58);§ mild impairment in adaptive skills	None
20	M	4 years	40·5 (5)	60·2 (7·5) at 4 years 4 months	1–2	Moderate ventriculomegaly, thin CC, prominent perivascular spaces, CSPV	Megalencephaly and polymicrogyria (in utero)	Focal seizures with unresponsiveness (15 months)	Hypotonia	Dysphagia, dysarthria, increased drooling	Mild ID, normal gross and fine motor skills	LGA

Patient research database numbers are provided in the supplementary methods of the appendix. Cognitive level was at last assessment unless otherwise stated. SD=OFC as standard deviation of means for age and sex.[6] BSID=Bayley Scales of Infant Development. CBTE=cerebellar tonsillar ectopia. CC=corpus callosum. CSPV=cavum septum pellucidum et vergae. F=female. FSIQ=full-scale intellectual quotient. GA=gestational age. GORD=gastro-oesophageal reflux disease. ID=intellectual disability. IQ=intelligence quotient. LD=learning disability. LGA=large for gestational age. M=male. OFC=occipitofrontal circumference. PIQ=Performance Intelligence Quotient. POI=Perceptual Organization Index. PSI=Processing Speed Index. VCI=Verbal Comprehension Index. VIQ=Verbal Intelligence Quotient. VSD=ventricular septal defect. WMI=Working Memory Index. ND=no data available. *This child is part of a large family of African-American ancestry that consists of 11 children, including five affected ones (appendix). Dysmorphic features seen in patient 7 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 study. †First available OFC at 10 months. ‡Wechsler Intelligence Scale for Children Revised (WISC-R). §Wechsler Adult Intelligence Scale Fourth Edition (WAIS-IV).

***Table 2*: Clinical and neuroimaging features of patients harbouring *PI3KR2* mutations**

and sex, fulfilling the diagnostic criteria for MPPH syndrome.[16] Megalencephaly was predominantly congenital in onset in these individuals, with later OFCs reported as large as 7·5 SD above the mean. Seven (35%) individuals were normocephalic.

Ventriculomegaly, ranging from mild to severe, was seen in 17 (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 were a variably thick corpus callosum (seven patients [35%]), cerebellar tonsillar ectopia (six [30%]), mild white matter dysmyelination with prominent perivascular spaces (seven [35%]), and cavum septum pellucidum et vergae (three [15%]; figures 2, 3).

Epilepsy occurred in 14 (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, apart from 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 only a few 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 than patients with mosaic mutations (mean 11 months *vs* 3·89 years).

	cDNA change	Aminoacid change	Germline or mosaic	Tissue tested	Testing method	Alternate allele fractions*	Inheritance
Constitutional *PIK3R2* mutations							
1	1117G→A	Gly373Arg	Germline	Blood	smMIPs, Sanger	115/262 (44%)	De novo
2	1117G→A	Gly373Arg	Germline	Blood	smMIPs, Sanger	171/388 (44%)	De novo
3	1117G→A	Gly373Arg	Germline	Blood; saliva	smMIPs, Sanger	Blood: 146/321 (45%); saliva: 132/316 (42%)	De novo
4	1117G→A	Gly373Arg	Possibly germline	Saliva	smMIPs, Sanger	125/251 (50%)	De novo
5	1117G→A	Gly373Arg	Germline	Blood	WES	Heterozygous†	De novo
6	1117G→A	Gly373Arg	Germline	Blood; saliva	Sanger	Blood: NA (50%); saliva: NA (50%)	De novo
7	1117G→A	Gly373Arg	Germline	Blood	smMIPs, Sanger	23/48 (48%)	Maternal
P (m)	1117G→A	Gly373Arg	Germline	Blood	smMIPs, Sanger	33/80 (41%)	NA
8	1117G→A	Gly373Arg	Possibly germline	Saliva	smMIPs, Sanger	102/219 (47%)	De novo
9	1117G→A	Gly373Arg	Germline	Saliva; blood	Saliva: smMIPs; blood: Sanger	Saliva: 33/71 (46%); blood: NA (50%)	De novo
10‡	1117G→A	Gly373Arg	Germline	Blood	Sanger	NA (50%)	Presumed parental germline mosaicism
11‡	1117G→A	Gly373Arg	Germline	Blood	Sanger	NA (50%)	Presumed parental germline mosaicism
P (f)	1117G→A	Gly373Arg	..	Blood	smMIPs	0/494	NA
P (m)	1117G→A	Gly373Arg	..	Blood	smMIPs	0/263	NA
12	1126A→G	Lys376Glu§	Germline	Blood	smMIPs, Sanger	111/197 (56%)	De novo
Mosaic PIK3R2 mutations							
13	1117G→A	Gly373Arg	Mosaic	Blood	smMIPs, Sanger	10/377 (7%)	De novo
14	1117G→A	Gly373Arg	Mosaic	Blood	Agilent SureSelect	41/778 (5%)	NA
15	1117G→A	Gly373Arg	Mosaic	Blood	smMIPs	36/493 (7%)	De novo
16	1117G→A	Gly373Arg	Mosaic	Blood	smMIPs	37/216 (17%)	NA
17	1117G→A	Gly373Arg	Mosaic	Saliva; blood	Saliva: smMIPs; blood: Sanger	Saliva: 17/53 (32%); blood: undetectable	De novo
18	1117G→A	Gly373Arg	Mosaic	Blood; saliva	Blood: WES; blood: amplicon sequencing; saliva: amplicon sequencing	Blood (WES): 10/86 (12%); blood (amplicon): 565/5453 (10%); saliva: 2030/6889 (29%)	De novo
19	1117G→A	Gly373Arg	Mosaic	Blood; saliva	Blood: WES; blood: amplicon sequencing; saliva: amplicon sequencing	Blood (WES): 20/132 (15%); blood (amplicon): 861/6449 (13%); saliva: 275/634 (43%)	De novo
20	1117G→A	Gly373Arg	Mosaic	Saliva; skin; blood; lipoma	smMIPs, Sanger	Saliva: 39/106 (37%); skin: 144/561 (26%); blood: 117/1052 (11%); lipoma: 1/7 (14%)	De novo

The genomic coordinates for these mutations are chromosome 19: 18273784G→A (Gly373Arg), and chromosome 19: 18273793A→G (Lys376Glu). The parents of patients 7, 10, and 11 were added to this table to show the alternate allele levels of the recurrent *PIK3R2* mutations as the parent of patient 7 harboured this mutation, whereas the parents of patients 10 and 11 tested negative, supporting possible germline mosaicism. NA=not available. P (f)=parent (father). P (m)=parent (mother). smMIPs=single molecule molecular inversion probes. WES=whole-exome sequencing. *Alternate allele fractions are based on the number of alternate or non-reference alleles/total alleles (%). †This patient underwent trio-based clinical WES. 99·5% of *PIK3R2* was covered at a minimum of 10×. Overall mean depth of coverage was 759×, with a quality threshold of 99·8%. ‡Poor DNA quality. Therefore, next-generation sequencing was not performed. No other tissue sources were available for analysis for this family. §This mutation is not present in any of the public databases (Single Nucleotide Polymorphism Database [dbSNP], 1000 Genomes, the National Heart, Lung, and Blood Institute [NHLBI] Exome Variant Server [EVS], the Exome Aggregation Consortium [ExAC]). It affects an evolutionarily conserved aminoacid residue and is predicted to be damaging by use of multiple in-silico prediction programmes (SIFT, PolyPhen-2, MutationTaster).

Table 3: **Mutations, levels of mosaicisim, and methods of detection of *PIK3R2* mutation-positive patients**

Symptoms of oromotor dysfunction such as expressive language or speech delay, difficulties handling oral secretions (such as profuse drooling), and dysphagia were present in most patients (nine [75%] of 12 patients with constitutional mutations and seven [88%] of eight patients with mosaic mutations).

Other notable manifestations were cutaneous capillary malformations (four patients), and multiple ventricular septal defects (one patient). Two patients had hypoglycaemia; in one (patient 5), it was transient at birth. The other (patient 14) had atypical ketotic hypoglycaemia at 6 years of age. Almost all of the patients had intellectual disability that varied from mild to severe. One patient with MPPH syndrome (patient 17) had early severe autistic features.

Constitutional *PIK3R2* mutations were de novo, apart from in two families (appendix). The first family (of patient 7) consisted of 11 children from multiple fathers, of whom five had megalencephaly, BPP, and variable hydrocephalus. One of these five children also had

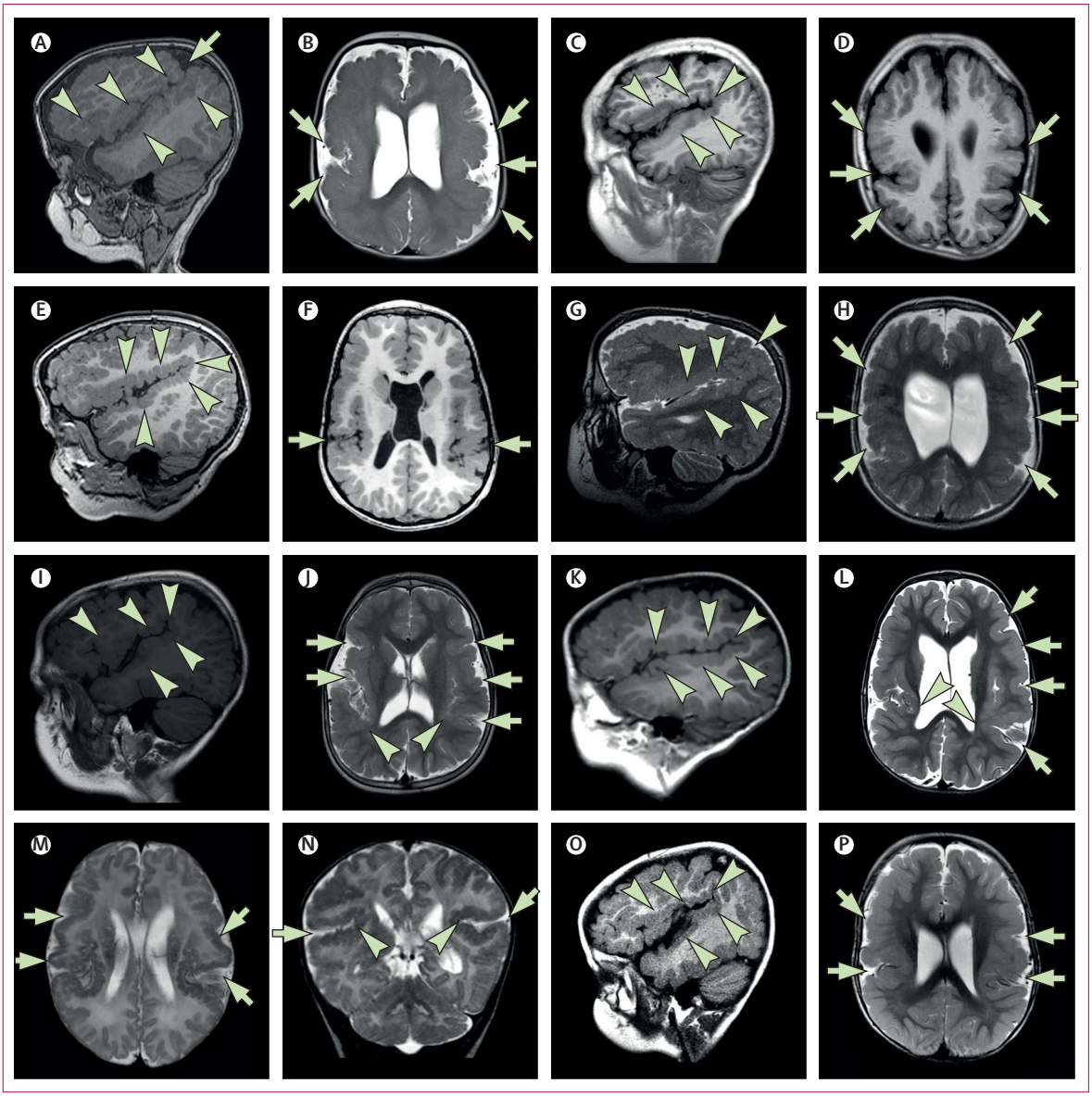

Figure 2: **Brain MRI scans of patients with constitutional *PIK3R2* mutations**
Representative T1-weighted and T2-weighted midsagittal, axial, and coronal 3-Tesla brain MRI images in patient 2 at age 2 years (A, B), patient 3 at age 8 years (C, D), patient 5 at age 5 years (E, F), patient 6 at age 3 years (G, H), patient 7 at age 2 years (I, J), patient 9 at age 18 months (K, L), patient 11 at age 21 days (M, N), and patient 12 at age 5 years (O, P). Note bilateral perisylvian polymicrogyria (arrows) and superiorly extended sylvian fissures (arrowheads). Other notable features include moderate to severe ventriculomegaly (B, F, H, L, and P) and cavum septum pellucidum et vergae (F, J, and M).

postaxial polydactyly, a known feature of MPPH syndrome. The mother had macrocephaly, hydrocephalus, intellectual disability, epilepsy, and schizoaffective disorder, but no brain imaging was available. Both child and mother harboured the *PIK3R2* mutation in peripheral blood-derived DNA at mutant allele levels of 23 (48%) of 48 reads and 33 (41%) of 80 reads, respectively, suggestive of maternal inheritance (table 3). Samples were not available from the other affected children. The second family (of patients 10 and 11) consisted 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 appendix.

Discussion

In this study, we report *PIK3R2* mutations in 20 children, consisting of 13 with MPPH syndrome and seven with BPP without megalencephaly. The *PIK3R2* mutations

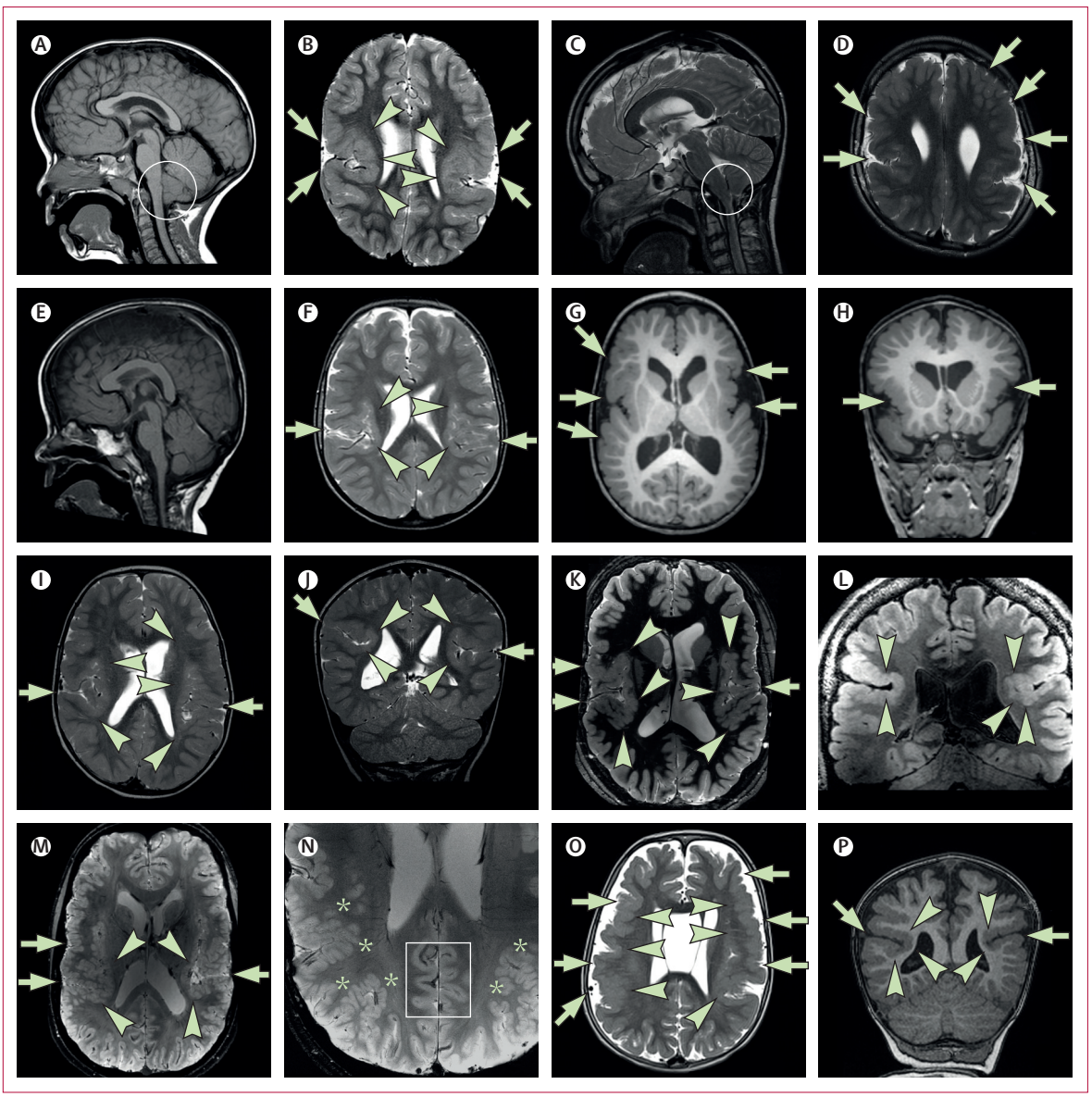

Figure 3: **Brain MRI scans of patients with mosaic *PIK3R2* mutations**
Representative T1-weighted and T2-weighted, SWAN, IR, 3-Tesla, and 7-Tesla midsagittal, axial, and coronal brain MRI images in patient 13 at age 4 years (A, B), patient 14 at age 12 years (C, D), patient 15 at age 2 years (E, F), patient 16 at age 3 years (G, H), patient 17 at age 2 years (I, J), patient 18 at age 14 years (K, L), patient 19 at age 22 years (M, N), and patient 20 at age 3 years (O, P). Note bilateral perisylvian polymicrogyria (arrows) and extended sylvian fissures (arrowheads). Images K, L, M, and N are at 7-Tesla. 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 to moderate ventriculomegaly (G, H, I, J, K, L, M, O), mild cerebellar tonsillar ectopia (A, C; white circles), thick corpus callosum (C, E), and cavum septum pellucidum et vergae (G, H, O). SWAN=susceptibility-weighted angiography. IR=inversion recovery.

identified in our cohort included de-novo constitutional mutations, mutations inherited from an affected parent or from parental germline mosaicism, and mosaic mutations. Our results show that mutations of this gene are associated with malformations of cortical development ranging from isolated BPP with a normal head size to BPP with megalencephaly, including MPPH syndrome.

BPP is the most common subtype of polymicrogyria and has been proposed to be an aetiologically heterogeneous anatomical and clinical syndrome, featuring a combination of oromotor dysfunction, cognitive impairment, and epilepsy.[1,17,18] Among the genetic causes, BPP has most often been reported in individuals with copy number variants, especially 1p36.3 and 22q11.2 deletion syndromes.[19,20] However, genetic heterogeneity has been proposed on the basis of reports of large families with possible autosomal dominant or X-linked inheritance with incomplete penetrance.[18,21] Polymicrogyria of variable

severity and distribution has been reported in many brain malformation syndromes caused by mutations in a growing number of genes (*NDE1, WDR62, OCLN, RAB3GAP1, RAB3GAP2, RAB18, DYNC1H1, KIF5C, EOMES*, *RTTN*, *FH*, *KIF1BP*), as well as many of the tubulin genes (*TUBA1A, TUBA8, TUBB2B, TUBB3, TUBB*).[1] However, polymicrogyria has been neuropathologically demonstrated for the *TUBA1A*, *TUBB2B*, and *OCLN* genes only.[22–24] For malformation syndromes related to the remaining genes, the defining characteristics of polymicrogyria, which are typically microscopic (many small microgyri, formed by thinned cortex, fused together), have been inferred on the basis of 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, despite similar imaging features.

Mutations of genes within the phosphatidylinositol-3-kinase (PI3K)-AKT-mTOR pathway are known to cause a wide range of developmental brain and body disorders. Specifically, mutations of *PIK3CA, PIK3R2, PTEN, AKT3*, and *CCND2* have been associated with focal, segmental (multifocal), and generalised megalencephaly with variable other features (appendix).[14,25–27] *PIK3R2* mutations specifically cause the MPPH syndrome,[14,16] a rare developmental brain disorder characterised by megalencephaly, polymicrogyria, ventriculomegaly often leading to hydrocephalus, and postaxial polydactyly.[16,28–31] Until now, mutations of *PIK3R2* have been reported in 15 individuals with this syndrome.[14,32,33] Mutations in two additional core pathway genes, *AKT3* and *CCND2*, have also been associated with MPPH.[14,27] Although 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 until now.[34]

Our data show that mutations of *PIK3R2* are an important cause of BPP, which otherwise remains aetiologically heterogeneous. Overall, constitutional and mosaic *PIK3R2* mutations accounted for 19 (15%) of our cohort of 126 patients with polymicrogyria, with mosaic mutations accounting for 6% of the cohort (n=8). This proportion is higher than that reported for most malformations of cortical development.[1,34] 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 seen in patients with constitutional mutations compared with patients with mosaic mutations.

PIK3R2 encodes the p85b regulatory subunit of the PI3K-AKT-mTOR pathway. The mutational spectrum is very narrow because all but one of the reported patients harboured the same missense mutation, Gly373Arg. This gain of function mutation lies within the sequence homology domain of the gene and is seen infrequently in somatic tissues in cancer.[35] Our data therefore expand on the phenotypic range 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* (Lys376Glu) in a girl with 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. Phenotypic brain involvement ranges from bilateral diffuse megalencephaly with normal gyral pattern to megalencephaly with polymicrogyria to hemimegalencephaly to focal cortical dysplasia type 2 (appendix).[14,25–27,36,37]

Our findings show that mosaic mutations of *PIK3R2* cause a regional brain malformation, similar to our experience with *PIK3CA*.[14] Although 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 hypothesise 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 fifth intrauterine month and completes opercularisation after birth.[38] The closure of the frontal and temporal opercula over the insula is among the most complex morphological changes occurring in the postembryonic cerebral hemispheres.[39] Deviations in cortical growth caused by 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.[38,40] However, it remains difficult to determine to what extent a regional brain malformation such as perisylvian polymicrogyria results from enhanced local vulnerability because of 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 patients with BPP, it is possible that other mosaic mutations in this cohort were missed because of either poor coverage or very low level mosaicism. We speculate that this is unlikely because the mean depth of coverage across our exomes was 141× and full coverage of *PIK3R2* coding exons was checked for our eight trios. Further, because we used a site-specific method (amplicon sequencing) to screen our cohort of 80 patients with polymicrogyria, we might have missed other mutations within the *PIK3R2* gene in this group. Another potential limitation of our findings is that the study is based mainly on analysis of DNA extracted from peripheral tissues (blood and saliva), and brain tissues were not accessible to detect or confirm mosaic

mutations. We expect that future next-generation sequencing studies of additional patients will further delineate the frequency of *PIK3R2* mutations in polymicrogyria in general, and for BPP in particular. Finally, similar to other studies, our results increase the known number of families with possible germline mosaicism. The role of germline mosaicism (ie, mosaic mutations in the germline cells of a parent) is increasingly being recognised as the cause of genetic disorders.[41,42] We expect that the frequency of germline mosaicism in disorders related to mutations in *PIK3R2* in particular will be further defined with future next-generation sequencing studies.

Contributors

GMM, RG, and WBD designed the study. GMM, SC, VC, BM, DM, and LT performed the genetic experiments. GMM, VC, AET, EAB, DM, J-FD, PN, JS, and JC analysed the molecular data. GMM, CDS, SA, MC, SB, RBH, AG, YN-K, CO, KJ, KF, CH, AG, EP, AS, EB, CB, WBD, and RG recruited and assessed the study participants. CO provided administrative support and recruited study participants, GMM, RG, and WBD supervised the study. GMM and RG wrote the manuscript.

Declaration of interests

JS has a patent for MIPgen Design Software with royalties paid to Roche. EAB has a patent for systems, algorithms, and software for molecular inversion probe (MIP) design with royalties paid to Integrated DNA Technologies. All other authors declare no competing interests.

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