

# BRAIN COMMUNICATIONS

## Genetic heterogeneity of polymicrogyria: study of 123 patients using deep sequencing

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Polymicrogyria is a malformation of cortical development characterized by overfolding and abnormal lamination of the cerebral cortex. Manifestations include epilepsy, speech disturbance and motor and cognitive disability. Causes include acquired prenatal insults and inherited and *de novo* genetic variants. The proportion of patients with polymicrogyria and a causative germline or mosaic variant is not known. The aim of this study was to identify the monogenic causes of polymicrogyria in a heterogeneous cohort of patients reflective of specialized referral services. Patients with polymicrogyria were recruited from two clinical centres in Australia and Belgium. Patients with evidence of congenital cytomegalovirus infection or causative chromosomal copy number variants were excluded. One hundred and twenty-three patients were tested using deep sequencing gene panels including known and candidate genes for malformations of cortical development. Causative and potentially causative variants were identified and correlated with phenotypic features. Pathogenic or likely pathogenic variants were identified in 25/123 (20.3%) patients. A candidate variant was identified for an additional patient but could not be confirmed as *de novo*, and therefore it was classified as being of uncertain significance with high clinical relevance. Of the 22 dominant variants identified, 5 were mosaic with allele fractions less than 0.33 and the lowest allele fraction 0.09. The most common causative genes were *TUBA1A* and *PIK3R2*. The other eleven causative genes were *PIK3CA*, *NEDD4L*, *COL4A1*, *COL4A2*, *GPSM2*, *GRIN2B*, *WDR62*, *TUBB3*, *TUBB2B*, *ACTG1* and *FH*. A genetic cause was more likely to be identified in the presence of an abnormal head size or additional brain malformations suggestive of a tubulinopathy, such as dysmorphic basal ganglia. A gene panel test provides greater sequencing depth and sensitivity for mosaic variants than whole exome or genome sequencing but is limited to the genes included, potentially missing variants in newly discovered genes. The diagnostic yield of 20.3% indicates that polymicrogyria may be associated with genes not yet known to be associated with brain malformations, brain-specific somatic mutations or non-genetic causes.

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Received August 3, 2020. Revised October 23, 2020. Accepted October 27, 2020. Advance Access publication December 26, 2020

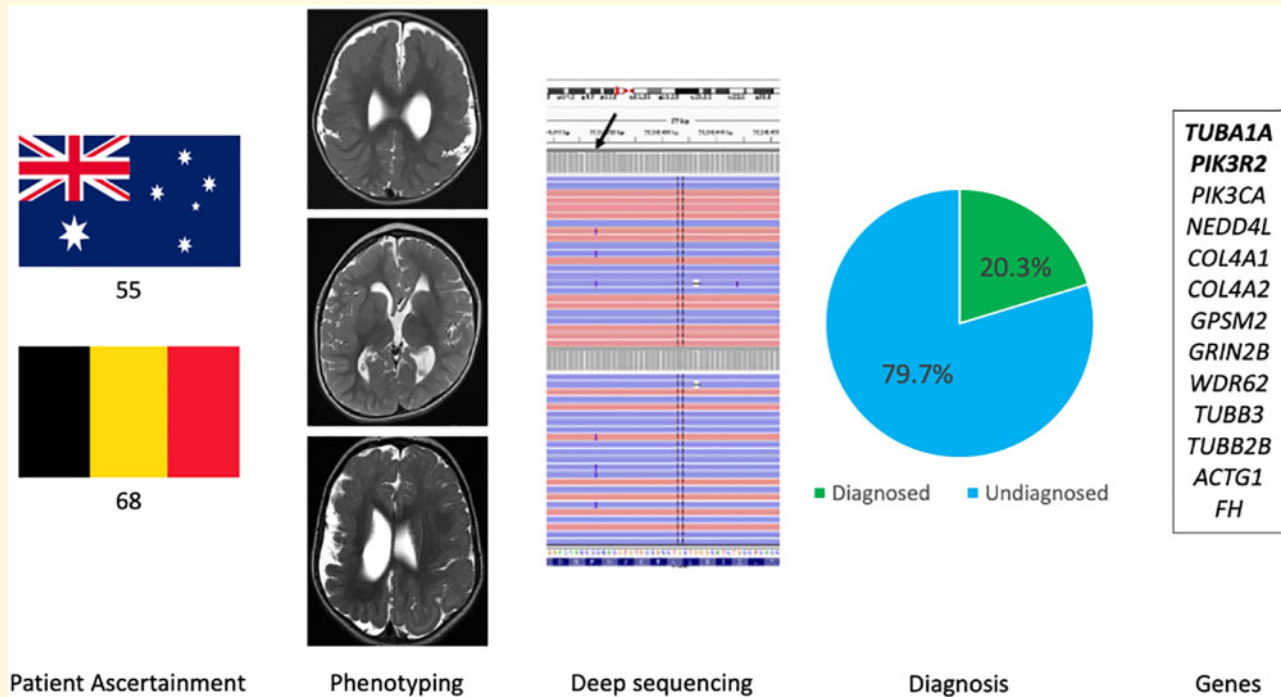
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**Keywords:** cortical malformation; epilepsy; genetic testing; high-throughput nucleotide sequencing; somatic mutation

**Abbreviations:** AF = allele fraction; AKT = protein kinase B; BGP = bilateral generalized polymicrogyria; bp = base pairs; BPP = bilateral perisylvian polymicrogyria; HC = head circumference; hg19 = human genome assembly 19 (GRCh37) from Genome Reference Consortium; LOVD = Leiden Open Variation Database; Mb = megabases; MCD = malformation of cortical development; MIM = Mendelian inheritance of man; mTOR = mammalian target of rapamycin; PI3K phosphatidylinositol 3-kinase; PMG = polymicrogyria; PNH-PMG = polymicrogyria associated with periventricular nodular heterotopia; RCH = Royal Children's Hospital, Melbourne, Australia; TRD = tubulin-related dysgyria; UZ = Universitair Ziekenhuis (University Hospital Brussels)

## Graphical Abstract



## Introduction

Polymicrogyria (PMG) is a malformation of the developing brain characterized by abnormal lamination and excessive folding of the cerebral cortex. It is one of the most common brain malformations and accounts for ~20% of all malformations of cortical development (Leventer *et al.*, 1999). PMG often occurs in association with other brain malformations and/or multiple congenital anomalies (Jansen and Andermann, 2005). Histologically, PMG is characterized by one or more undulating or festooning neuronal layers replacing the normal six-layered cortical architecture and is frequently associated with thickened leptomeninges (Jansen *et al.*, 2016). In practice, the diagnosis is usually based on neuroimaging, the MRI features of which are an irregular, bumpy appearance of the cortex with apparent cortical thickening and a stippled grey-white matter junction (Leventer *et al.*, 2010). The clinical consequences depend on the extent and regions of the brain affected, associated congenital malformations in the

brain or elsewhere, the underlying aetiology and comorbidities. Common manifestations include epilepsy, intellectual disability, cerebral palsy and oro-motor impairment.

The aetiologies of PMG are heterogeneous and include acquired and genetic causes. It has been proposed that PMG is the common endpoint of many different aetiological processes occurring at specific stages in cortical development (Jansen *et al.*, 2016). Acquired causes include congenital cytomegalovirus infection (Crome and France, 1959) and foetal cerebral ischaemia (Levine *et al.*, 1974). Genetic causes include chromosomal and monogenic variants, the latter of which may be inherited or *de novo* and can be germline or mosaic. PMG is morphologically heterogeneous, and an identical morphology does not necessarily imply similar aetiology which makes diagnosis of the underlying cause challenging. There are different methods of classification of PMG. It was initially based on the histological appearance (Friede, 1989). Subsequently, it was classified according to imaging patterns (Leventer *et al.*, 2010) and the presumed timing of

the aetiology in cortical development (Barkovich *et al.*, 2012). Now that mutations in at least 50 genes have been associated with PMG, these causes can be classified according to the molecular pathways implicated in the aetiology (Stutterd *et al.*, 2018). This recent approach to the classification of cases with suspected genetic aetiology is useful in clinical practice as it can inform genetic testing and reproductive counselling.

Previous genetic studies of PMG have interrogated the contribution of mutations in specific genes or pathways in patient groups with common phenotypic features such as mammalian target of rapamycin (mTOR) pathway mutations in patients with PMG and megalencephaly (Mirzaa *et al.*, 2015) or screening patient cohorts for mutations in tubulin genes (Bahi-Buisson *et al.*, 2014). The proportion of patients with PMG with an identifiable germline or mosaic variant is not known and this clinical question was the motivation for our study. We performed genomic analysis of two phenotypically heterogeneous cohorts from two centres totalling 123 patients. A chromosomal cause and congenital cytomegalovirus (if indicated) were excluded where possible, reflective of clinical practice. We tested for germline and mosaic variants in all genes known to be associated with PMG at the time of the study to establish the diagnostic yield and contribution from mosaic mutations and identify any genotype–phenotype correlations.

## Materials and methods

### Patient recruitment

Patients were ascertained from the Royal Children's Hospital (RCH) Melbourne Brain Malformation Research Database and the Center of Medical Genetics, Universitair Ziekenhuis (University Hospital Brussels) Brussel Brain Malformation Database via protocols approved by the RCH Human Research Ethics Committee and the Institutional Review Board at Universitair Ziekenhuis (University Hospital Brussels) Brussels. Patients were included if there was PMG identified on MRI or if they had been referred for genetic testing for the indication of PMG. Patients were excluded if there was evidence of congenital cytomegalovirus infection from neonatal serology or retrospective testing of a dried blood spot sample from their Newborn Screening Card. Patients were excluded if they had a causative copy number variant detected by chromosome microarray. Of the 123 patients included, 55 were recruited from Australia and 68 from Belgium. Results of non-diagnostic chromosome microarray were available for 63/123 patients (55 Australian, 8 Belgium). Congenital cytomegalovirus was excluded for 40/123 patients (34 Australian, 6 Belgium) and testing for this was not clinically indicated for 15/123 (Australian cohort) based on their clinical phenotype.

## Clinical and imaging phenotyping

Patient records were reviewed for information regarding sex, family history, prenatal risk factors (twinning, antepartum haemorrhage or infection), head circumference (HC), presence and type of congenital malformation and presence of seizures. All available MRIs (for 112 patients) were reviewed by the paediatric neurologist supervising the project at each site (RJL at RCH and AJ at Universitair Ziekenhuis (University Hospital Brussels) Brussel). The distribution of PMG was recorded as one of the six recurrent topographic patterns as described by Leventer *et al.* (Leventer *et al.*, 2010): bilateral perisylvian polymicrogyria (BPP), unilateral perisylvian polymicrogyria, bilateral generalized polymicrogyria (BGP), bilateral frontal polymicrogyria, bilateral frontoparietal polymicrogyria, bilateral parasagittal parieto-occipital polymicrogyria or polymicrogyria associated with periventricular nodular heterotopia in addition to patterns consistent with schizencephaly (unilateral or bilateral) or 'tubulin-related dysgyria' (TRD). The latter term describes a pattern of cortical malformation seen in association with disordered tubulin function in which there is an abnormal gyral pattern characterized by abnormal sulcal depth or orientation and narrow gyri separated by abnormally deep or shallow sulci (Oegema *et al.*, 2015; Mutch *et al.*, 2016) but is often reported as PMG. The presence and type of additional brain malformations were recorded.

## Genomic analysis

Genomic analysis was conducted at two sites using two different gene panels. The Australian cohort was tested at Murdoch Children's Research Institute (MCRI) using a custom in-house panel targeting 328 genes including confirmed and candidate genes associated with brain malformations. The Belgium cohort was tested at the Center of Medical Genetics, Universitair Ziekenhuis (University Hospital Brussels) Brussel with a custom gene panel targeting 190 genes associated with malformations of cortical development. There were 141 genes common to both gene lists. The panel gene lists and overlapping genes are shown in [Supplementary File 1](#). Of the 50 genes associated with PMG in the GeneReviews Polymicrogyria Overview updated in 2018 (Stutterd *et al.*, 2018), 43 and 47 are included in the Australian and Belgian panels respectively and 42 in the overlapping gene list (highlighted in bold in each gene list in [Supplementary File 1](#)).

Australian cohort: A custom in-house gene panel that targets 328 genes associated with brain malformations was developed using the HaloPlexHS target enrichment system (Agilent Technology). This panel was validated to detect mosaic variants with a minor allele frequency above or equal to 2% (Lee *et al.*, 2019, 2020). Fifty nanograms of genomic DNA from blood or saliva was utilized to prepare the sequencing library according to

the manufacturer's instructions and  $2 \times 150$  bp paired-end sequencing to a median depth of  $>600\times$  was performed on a NextSeq (Illumina). The raw data files (fastq.gz) were analysed using both SureCall software (Agilent Technology) and Leiden Open Variation Database v.3.0 build 17 software (Leiden University Medical Center). The GRCh37/hg19 version of the human reference genome assembly was used for read alignment. Variants were filtered out based on quality ( $QUAL < 50$ ), population frequency ( $>1\%$ , The Exome Aggregation Consortium), region (deep intronic or untranslated regions) and type (synonymous). High-frequency variants within our cohort of cases were used to discard variants likely due to a sequencing artefact. Variant prioritization was performed following two parallel approaches, each of them blind to the other, a clinically driven approach, previously described by [Sadedin et al. \(2015\)](#) and a molecular-driven approach ([Supplementary File 2](#)). Variants prioritized by both methods were then further evaluated by a curation team composed of a clinical geneticist, a neurologist and a senior medical scientist. Candidate recessive variants and variants with an uncertain but high chance of clinical significance were segregated to inform pathogenicity.

**Belgian cohort:** Patients with malformations of cortical development were analysed by the 'MCD gene panel', a custom gene panel that targets 190 genes associated with malformations of cortical development. The gene content of the panel is listed in [Supplementary File 1](#). This analysis was performed at the Center of Medical Genetics, Universitair Ziekenhuis (University Hospital Brussels) Brussel in collaboration with the Brussels Interuniversity Genomics High Throughput core according to standard procedures with an average coverage of  $500\times$ . In short, 0.5 mg deoxyribonucleic acid (DNA) was fragmented into pieces with an average length of 220 bp. A DNA library was prepared following the manufacturer's instructions (KAPA Biosystems). Next, five DNA libraries were captured by the use of Roche Nimblegen SeqCap EZ Choice XL enrichment probes. These fragments were amplified on the Illumina cBot machine and sequenced on the Illumina HiSeq1500 instrument or on the Illumina Novaseq 600 machine. Raw data were quality-controlled by use of FastQC (v0.10.1) and mapped to the human reference genome (hg19) with BWA 0.7.10. Mapping qualities were assessed via overall coverage analysis by an in-house designed script. The mapped reads were processed using the GATK 2.7 (Genome Analysis Toolkit) pipeline (IndelRealigner, BaseRecalibrator, HaplotypeCaller) and the detected variants were annotated by Annovar or Alamut Batch.

## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its [Supplementary material](#).

# Results

## Clinical features

One hundred and twenty-three patients were included in the study. The sex was known for 121 patients, 60% of whom were male. There were four familial cases in the cohort, all involving two affected siblings, and the remaining 115 cases were sporadic. Four patients were assessed and tested prenatally as foetuses and the remaining 119 patients were children or adults. Eight patients were born from a twin pregnancy, three dizygotic, two monozygotic and three with unknown zygosity. One had a twin sibling who died *in utero* at four months gestation and seven had an unaffected twin sibling. Ninety-five of 123 patients had a HC documented at the time of genetic testing. Of those with a recorded HC, the majority were normocephalic (60%), 27.3% were microcephalic ( $<2$  standard deviation from mean) and 12.6% were macrocephalic ( $>2$  standard deviation from mean). The presence or absence of congenital malformations outside of the central nervous system was documented in 77 patients and were present in 40 (51.9%), 10 of whom had more than one non-central nervous system malformation. Recurrent malformations were facial dysmorphism, eye anomalies (blepharophimosis, ptosis, strabismus, abnormal palpebral fissures, microphthalmia, cataract and retinal dystrophy), limb malformations (arthrogryposis, talipes), capillary malformation, syndactyly, vocal cord palsy and sensorineural hearing loss. Malformations documented in single cases only were polydactyly, hemihypertrophy, short stature, hypertrichosis and hypospadias. The presence or absence of epilepsy was documented for 91 patients, for whom it was present in 59.3%.

## PMG distribution

Imaging or information on PMG distribution was available for 112 patients. Forty-four of 112 (39.2%) had bilateral perisylvian polymicrogyria. The next most common distribution patterns were unilateral perisylvian polymicrogyria and BGP seen in 18/112 (16%) and 17/112 (15.2%), respectively. All of the other six distribution patterns were identified amongst the remaining 33 cases. Tubulin-related dysgyria was identified in five patients who had been referred for genetic testing based on an MRI diagnosis of PMG. A review of these MRIs after recruitment to the study led to re-classification of the malformations of cortical development as tubulin-related dysgyria.

## Additional brain malformations

Numerous additional brain malformations were identified. The most common were periventricular nodular heterotopia, abnormalities of the corpus callosum (complete or partial absence, thin, thick, dysplastic, elongated),

abnormal septum pellucidum (cavum, absence or partial absence), abnormalities of the basal ganglia, brainstem and cerebellum (hypoplasia, dysplasia, asymmetry), calcifications (cortical, subcortical and thalamic), white matter abnormalities, dysplastic ventricles, subcortical heterotopia and porencephalic cyst. Less common abnormalities reported in a single patient only (some in the same patient) were under-rotated hippocampi, hemimegalencephaly, cerebellar cysts, hydrocephalus, fused colliculi, pineal mass, Chiari I malformation, enlarged extra-axial spaces, posterior PNH, optic nerve hypoplasia and choroid plexus cysts.

## Genetic causes

A pathogenic or likely pathogenic variant was identified in 25/123 (20.3%) patients and a variant of uncertain significance with high clinical relevance was identified in one patient. Of the 25 pathogenic or likely pathogenic variants, 14 were from the Australian cohort and 11 from the Belgium cohort. One patient from the Belgium cohort was identified to have a variant of uncertain significance with high clinical relevance. The variants identified in these 26 patients involved 13 different genes (Table 1) and included autosomal recessive variants, inherited dominant variants and *de novo* dominant variants, both germline and mosaic. No X-linked variants were identified. The most commonly implicated genes were *TUBA1A* (6/26) and *PIK3R2* (5/26). Causative variants were also identified in *PIK3CA*, *NEDD4L*, *COL4A1*, *COL4A2*, *GPSM2*, *GRIN2B*, *WDR62*, *TUBB3*, *TUBB2B*, *FH* and *ACTG1*. Of the 22 dominant variants, five were likely mosaic variants based on their low allele fractions (AFs) and these were identified in two genes, *PIK3R2* and *PIK3CA*. The lowest AF was 0.09 detected for a *PIK3CA* variant in a blood-derived DNA sample. Additional genotypic and phenotypic information relating to each variant is provided in Supplementary Table 1. Representative brain MR images from patients with six of the most commonly identified genes are shown in Fig. 1.

## Tubulinopathies

Eight of the 26 (30.7%) causative or candidate variants were in tubulin-related genes, six in *TUBA1A* and one each in *TUBB2B* and *TUBB3*. Parental testing was available for seven of the eight variants and the variant was shown to be *de novo* in each case. Parental testing was not available for one variant in *TUBA1A*, which is a novel missense variant and therefore remains classified as a variant of uncertain significance. As *TUBA1A* is highly intolerant to missense variation (observed number of variants in population is 0.7 compared with expected number of 260.7;  $Z$  score = 5.58) (Lek *et al.*, 2016) and there is a strong phenotype correlation in this case, the variant would be reclassified as 'likely pathogenic' if

confirmed *de novo*. None of the *TUBA1A* variants were recurrent within the cohort and all were missense. Three were previously reported variants and three were novel. The genotype–phenotype correlations identified for this group are discussed below.

## mTORopathies

The second most frequently implicated molecular pathway was the mTOR-PI3K-AKT pathway, with causative variants in the *PIK3R2* or *PIK3CA* genes identified in 7 of 26 (26.9%) patients with a causative or candidate variant(s) identified. The recurrent *PIK3R2* variant, p.Gly373Arg was identified in five patients and this variant alone constituted 20% of the causative and candidate variants identified in the entire cohort. The AF for the *PIK3R2* variants ranged from 0.14 to 0.5. *PIK3CA* variants were identified in two patients and were mosaic in both cases. For one patient, the variant was detectable only in the patient's skin sample (AF 0.3) and was not detected in their blood sample (see Table 1 Pt AJ52: p.Pro449Ser). The second *PIK3CA* variant was detected with a similar AF in the patient's blood (AF 0.09) and saliva (AF 0.11) samples. This was the lowest AF for a causative variant detected in this study.

## Collagenopathies

Three patients from two families were diagnosed with collagenopathies with causative variants identified in *COL4A1* and *COL4A2*. We identified a novel, maternally inherited splice variant in *COL4A2* (c.2902+1G>A) in two affected sisters, one with intellectual disability and schizencephaly and the other with PMG, epilepsy and normal cognitive function. Family segregation studies showed that the variant was inherited from their mother who had died from small vessel cerebral disease and therefore it was classified as likely pathogenic. We identified a novel, *de novo* *COL4A1* variant (p.Gly948Arg) in a female with microcephaly, cerebral palsy and BPP, porencephaly and multifocal haemorrhagic infarctions. Evidence of cerebrovascular accident had been identified on antenatal ultrasound at 35 weeks' gestation. This is a novel variant causing substitution of a glycine residue in the triple amino acid repeat sequence of the triple-helical domain which is a common mutational mechanism for collagenopathies.

## Other autosomal dominant causes

Two unrelated patients with periventricular nodular heterotopia and syndactyly were identified with a recurrent heterozygous variant in *NEDD4L* (p.Arg208Gln), inherited from an affected parent in one case and *de novo* in the other. These patients have been published separately by Stouffs *et al.* (2020). A *de novo* variant in *GRIN2B* (p.Leu813Val) was identified in one patient with microcephaly, epilepsy and BGP with a thin corpus callosum.

**Table 1 Genetic results for patients with causative variant identified**

Patient ID	Gene	Causative variant	Allele fraction <sup>a</sup> (tissue)	Inheritance	Variant class	PMG distribution	Head size
CS34	<i>ACTG1</i>	NM_001614.5( <i>ACTG1</i> ):c.616C>T; p. Arg206Trp	Het	<i>De novo</i>	LP	BPP	Norm
CS53	<i>COL4A1</i>	NM_001845.6( <i>COL4A1</i> ):c.2842G>C; p. Gly948Arg	Het	<i>De novo</i>	LP	BPP	MIC
CS40	<i>COL4A2</i>	NM_001846.4( <i>COL4A2</i> ):c.2902 + 1G>A	Het	Maternal (sibling of CS41)	LP	UPP	Norm
CS41	<i>COL4A2</i>	NM_001846.4( <i>COL4A2</i> ):c.2902 + 1G>A	Het	Maternal (sibling of CS40)	LP	SCZ	Norm
AJ34	<i>FH</i>	NM_000143.3( <i>FH</i> ):c.1169A>G, p. Asn390Ser	Hom	Bi-parental	LP	BGP	NR
CS7	<i>GPSM2</i>	NM_013296.5( <i>GPSM2</i> ):c.1501delA; Ser501Alafs*30	Hom	Bi-parental	LP	BGP	Norm
AJ42	<i>GRIN2B</i>	NM_000834.4( <i>GRIN2B</i> ):c.2437C>G, p. Leu813Val	Het	<i>De novo</i>	LP	BGP	MIC
AJ2	<i>NEDD4L</i>	NM_001144967.3( <i>NEDD4L</i> ):c.623G>A, p. Arg208Gln	Het	Maternal	LP	BPP (PNH-PMG)	Norm
AJ15	<i>NEDD4L</i>	NM_001144967.3( <i>NEDD4L</i> ):c.623G>A, p. Arg208Gln	Het	<i>De novo</i>	LP	BPP (PNH-PMG)	MAC
CS10	<i>PIK3R2</i>	NM_005027.3( <i>PIK3R2</i> ):c.1117G>A; p. Gly373Arg	0.14 (blood)	Mosaic	P	BPP	Norm
CS27	<i>PIK3R2</i>	NM_005027.3( <i>PIK3R2</i> ):c.1117G>A; p. Gly373Arg	0.14 (blood)	Mosaic	P	BPP	MAC
CS46	<i>PIK3R2</i>	NM_005027.3( <i>PIK3R2</i> ):c.1117G>A; p. Gly373Arg	0.22 (saliva)	Mosaic	P	BGP	MAC
CS51	<i>PIK3R2</i>	NM_005027.3( <i>PIK3R2</i> ):c.1117G>A; p. Gly373Arg	Het	Unknown	P	BFP	MAC
AJ57	<i>PIK3R2</i>	NM_005027.3( <i>PIK3R2</i> ):c.1117G>A; p. Gly373Arg	Het	<i>De novo</i>	P	BGP	MAC
AJ52	<i>PIK3CA</i>	NM_006218.4( <i>PIK3CA</i> ):c.1345C>T, p. Pro449Ser	0.30 (skin) 0.00 (blood)	Mosaic	LP	BPP	MAC
CS3	<i>PIK3CA</i>	NM_006218.4( <i>PIK3CA</i> ):c.2740G>A, p. Gly914Arg	0.09 (blood) 0.11 (saliva)	Mosaic	P	BPP	MAC
AJ5	<i>TUBA1A</i>	NM_006009.4( <i>TUBA1A</i> ):c.5G>A, p. Arg2His	Het	<i>De novo</i>	P	BPP	MIC
CS32	<i>TUBA1A</i>	NM_006009.4( <i>TUBA1A</i> ):c.598T>C, p. Cys200Arg	Het	<i>De novo</i>	LP	BPP	MIC
CS37	<i>TUBA1A</i>	NM_006009.4( <i>TUBA1A</i> ):c.746A>G, p. Asn249Ser	Het	<i>De novo</i>	LP	TRD	MIC
AJ22	<i>TUBA1A</i>	NM_006009.4( <i>TUBA1A</i> ):c.958C>T, p. Arg320Cys	Het	<i>De novo</i>	P	Unknown	NR
AJ58	<i>TUBA1A</i>	NM_006009.4( <i>TUBA1A</i> ):c.1168C>T, p. Arg390Cys	Het	<i>De novo</i>	P	TRD	MIC
AJ46	<i>TUBA1A</i>	NM_006009.4( <i>TUBA1A</i> ):c.1182G>C, p. Lys394Asn	Het	Unknown	VUS	TRD	Norm
AJ43	<i>TUBB2B</i>	NM_178012.5( <i>TUBB2B</i> ):c.602G>T, p. Cys201Phe	Het	<i>De novo</i>	LP	TRD	NR
AJ68	<i>TUBB3</i>	NM_006086.4( <i>TUBB3</i> ):c.292G>A, p. Gly98Ser	Het	<i>De novo</i>	LP	BPP	Norm
CS19	<i>WDR62</i>	NM_001083961.2( <i>WDR62</i> ):c.836G>A, p. Cys279Tyr	Het	Paternal	LP	BGP	MIC
CS20	<i>WDR62</i>	NM_001083961.2( <i>WDR62</i> ):c.1480G>A, p. Gly494Arg	Het	Maternal	LP		
		NM_001083961.2( <i>WDR62</i> ):c.836G>A, p. Cys279Tyr	Het	Paternal	LP	BPP	MIC
		NM_001083961.2( <i>WDR62</i> ):c.1480G>A, p. Gly494Arg	Het	Maternal	LP		

BFP = bilateral frontal polymicrogyria; BG = basal ganglia; BGP = bilateral generalized polymicrogyria; BPP = bilateral perisylvian polymicrogyria; CC = corpus callosum; Comp het = compound heterozygous; CSP = cavum septum pellucidum; Het = heterozygous; Hom = homozygous; LP = likely pathogenic; MAC = macrocephaly; MIC = microcephaly; Norm = normal; NR = No record; P = pathogenic; PIPO = paediatric intestinal pseudo-obstruction; PNH-PMG = polymicrogyria associated with periventricular nodular heterotopia; UPP = unilateral perisylvian polymicrogyria; VUS = variant of uncertain significance.

<sup>a</sup>The AF is provided for mosaic cases only (variants with AF <0.33).

A novel, *de novo* variant in *ACTG1* (p.Arg206Trp) was identified in a child with BPP without the facial dysmorphism that is typical for Baraitser–Winter syndrome. This finding expands the phenotypic spectrum of *ACTG1* to non-syndromic cortical malformation and expands the spectrum of associated cortical malformation to include PMG.

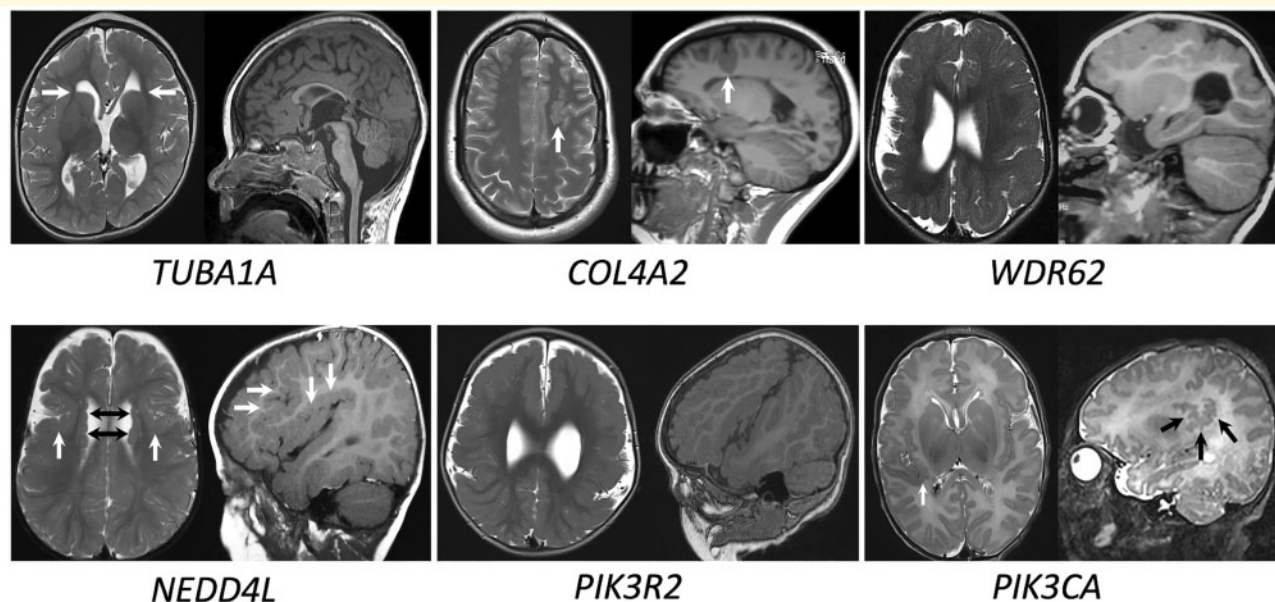
### Autosomal recessive causes

Compound heterozygous variants in *WDR62* (p.Cys279Tyr and p. Gly494Arg) were identified in two similarly affected sisters with microcephaly, epilepsy and cerebral palsy. A novel, homozygous frameshift variant in *GPSM2* (Ser501Alafs\*30), was identified in a child born to consanguineous parents with treated hydrocephalus, congenital sensorineural hearing loss and bilateral frontal parasagittal PMG with hypoplastic

corpus callosum, consistent with Chudley–McCullough Syndrome. A homozygous variant was identified in the gene encoding fumarate hydratase, *FH* (p.Asn390Ser) in one patient born to consanguineous parents with BGP and agenesis of the corpus callosum. For this patient, *FH* activity in fibroblasts and leucocytes was reduced to 25% and MRI findings are consistent with fumarate hydratase deficiency (Kerrigan *et al.*, 2000) providing evidence for likely pathogenicity of this variant for the PMG.

### Chromosomal variants of uncertain significance

Two patients included in the study have chromosome copy number variants of uncertain significance that may be relevant to their PMG. A monogenic cause for PMG was not identified in either patient and both patients had



**Figure 1 Representative MRI images for patients with different molecular diagnoses.** Images are T2 axial, T1 or T2 parasagittal. **TUBA1A** (patient CS37, aged 2 years at scan) shows an irregular, overfolded, thickened cortex particularly in the frontal regions. This cortical appearance is now described by some as 'tubulin-associated dysgyria'. The basal ganglia are dysmorphic, giving the frontal horns a 'hooked aspect' (white arrows). The corpus callosum is thin, the cerebellar vermis hypoplastic and brainstem dysmorphic with a flattened pons and bulky medulla. **COL4A2** (patient CS40, aged 40 years at scan) shows a deep sulcus in the left frontal lobe with PMG branching from its base (white arrow). **WDR62** (patient CS19, aged 15 months at scan) shows extensive right hemisphere PMG and a dysmorphic right lateral ventricle. **NEDD4L** (patient AJ15, aged 14 months at scan) shows bilateral PMG centred around the Sylvian fissures (white arrows) and underlying periventricular nodular grey matter heterotopia (black arrows). **PIK3R2** (patient CS10, aged 23 months at scan) shows generalized PMG with relative sparing of the medial occipital gyri. The Sylvian fissures are abnormally extended and oriented superiorly. **PIK3CA** (patient CS3, aged five days at scan) shows posterior perisylvian PMG on the right (white arrows). Other slices showed a similar appearance to the left posterior Sylvian fissure.

congenital cytomegalovirus excluded. One patient (CS55) has BPP and nephronophthisis and a homozygous deletion of *NPHP1*. This interstitial deletion involves  $\sim 0.12$  Mb at 2q13: arr[hg19]2q13(110 859 672–110 982 530) $\times 0$ . She has normocephaly and no additional brain or other malformations. This homozygous deletion is a known cause for nephronophthisis 1 (MIM#256100) but has never been reported before in association with PMG. At least one other gene associated with Joubert syndrome, *AHI1*, is known to cause both nephronophthisis and PMG (Dixon-Salazar *et al.*, 2004; Utsch *et al.*, 2006). Therefore, it is possible that the ciliopathy in this case is the cause for the patient's PMG however, another example of this has not been published. The second patient (CS36) is a male with BPP and a maternally inherited duplication of  $\sim 0.4$  Mb at Xq28: arr[hg19]Xq28(154 132 683–154 536 836) $\times 2$ . He has normocephaly and no additional brain or other malformation. The Xq28 duplication involves seven RefSeq genes including *RAB39B*. Duplications of this region, and *RAB39B* specifically, have been associated with intellectual disability but not cortical malformation to date (El-Hattab *et al.*, 2011; Vanmarsenille *et al.*, 2014).

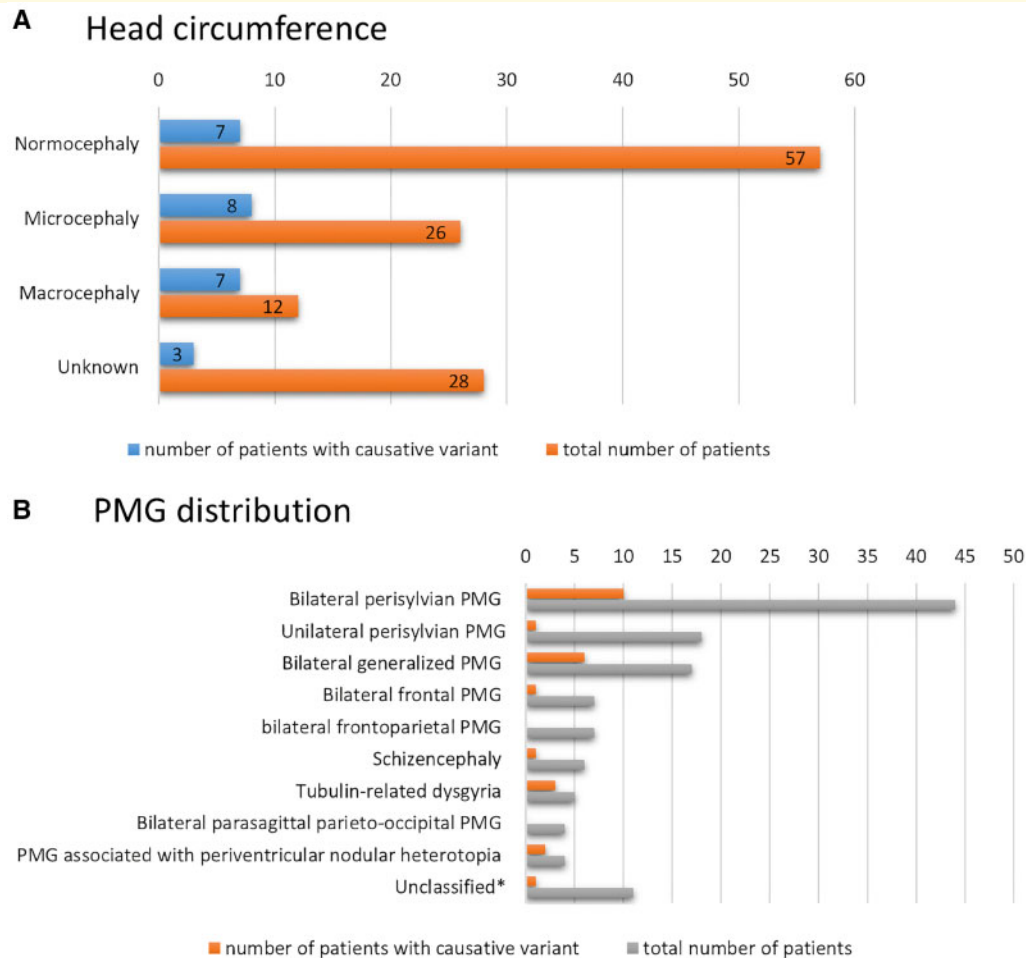
## Genotype–phenotype correlations

### Family history

The genetic causes identified were not significantly different between male (56%) and female (44%) patients. There were three sets of affected siblings and one set of half-siblings included in the study. A genetic cause was identified in three of these four families, compound heterozygous variants in *WDR62* for one family and inherited variants in *COL4A2* and *NEDD4L* for two. Parental consanguinity was reported for four patients, two of whom had homozygous causative variants affecting *GPSM2* and *FH*, respectively.

### Prenatal risk factors

Eight patients tested had been born of a twin pregnancy and a genetic cause was identified for only one of these patients. The cause in this dizygous twin was a *de novo* heterozygous *PIK3R2* variant p. Gly373Arg. A genetic cause was not identified in the other seven patients born of a twin pregnancy, two dizygotic, two monozygotic and two of unknown zygotic status.



**Figure 2 Graphical visualization of the diagnostic yield in patient subgroups. (A)** Graphical visualization of the diagnostic yield depending on the HC. **(B)** Graphical visualization of the diagnostic yield in each morphological subtype of PMG. \*Unclassified group refers to patients for whom MR images or detailed data was not available.

### Head circumference

The highest diagnostic yield was in patients with abnormal head growth, as recorded at the time of genetic testing. A genetic diagnosis was confirmed in 58.3% of patients with macrocephaly. The genes implicated in this group were *PIK3R2*, *PIK3CA* and *NEDD4L*. All patients with an mTORopathy had macrocephaly except one who was mosaic for the recurrent *PIK3R2* variant with an AF of 0.14 and had a HC within the normal range. Of the 26 patients in the cohort with microcephaly, a genetic cause was identified in eight, giving a diagnostic yield of 30.8% in this group. The genes implicated in this group were *TUBA1A*, *WDR62*, *GRIN2B* and *COL4A1*. The proportion of these patients who had an acquired rather than congenital abnormality in head growth is not known. The diagnostic yield according to HC is shown in Fig. 2A. The causative genes implicated according to head size are shown in Supplementary Table 2A.

### Additional congenital anomalies

PMG can be seen as a feature in numerous genetic syndromes (Stutterd and Leventer, 2014). Of the 40 patients with congenital malformation outside the central nervous system, 13 had a genetic cause identified (32.5%). Of the 37 patients with no congenital malformation outside the central nervous system, a genetic diagnosis was identified for 8 (21.6%). This is not significantly different. Of the 6/8 cases of tubulinopathy with records available regarding presence or absence of congenital malformation, five had eye abnormalities. These were documented as abnormal palpebral fissures in four cases, the abnormality of which was ptosis in two cases with blepharophimosis in one case. Two patients had strabismus. The presence of congenital malformations in association with an mTORopathy was variable and not all of these patients had features of the typically associated Megalencephaly-PMG-Polydactyly-Hydrocephalus or Megalencephaly-Capillary malformation and polymicrogyria syndromes. Six of the seven patients



had macrocephaly, but additional features were only seen in two; one with capillary malformation and polydactyly and one with capillary malformation only.

### PMG distribution

The PMG distribution was not predictive for diagnostic yield except in cases recognized as having the distribution now referred to as ‘tubulin-related dysgyria’ (Oegema *et al.*, 2015), for whom 4/5 patients had a causative or candidate tubulin gene variant identified (*TUBA1A* or *TUBB2B*). Outside of tubulin-related dysgyria, the PMG distribution was not predictive for genotype except in one patient who had the rare distribution pattern of frontal parasagittal PMG accompanied by ventriculomegaly and sensorineural hearing loss which is consistent with Chudley–McCullough syndrome and, in this patient, a causative homozygous variant in *GPSM2* was identified. The diagnostic yield according to PMG distribution is shown in Fig. 2B. The causative genes implicated according to PMG distribution are shown in Supplementary Table 2B.

### Additional brain malformations

The presence or absence of additional brain malformations did not correlate with diagnostic yield in this cohort. However, additional brain malformations were present in all patients diagnosed with a tubulinopathy. The additional brain malformations in these cases included agenesis/hypoplasia or the corpus callosum, dysplastic basal ganglia, brain stem hypoplasia and hypoplasia/dysplasia of the cerebellum/vermis, all of which are recognized malformations in tubulinopathies (Mutch *et al.*, 2016).

## Discussion

### Diagnostic yield

In this study, 123 individuals with PMG had sequencing on a NGS panel and a pathogenic or likely pathogenic variant was identified in 25 (20.3%), 14/55 patients in the Australian cohort and 11/68 in the Belgium cohort. A variant of uncertain significance with high clinical relevance was identified in one additional patient from the Belgium cohort. Of the 22 autosomal dominant variants identified, five were likely mosaic variants based on their low AF (<0.33) and the lowest AF was 0.09. The most commonly implicated genes were *TUBA1A* and *PIK3R2*. Several genotype–phenotype correlations were identified, highlighting the importance of clinical phenotyping for variant prioritization and interpretation. Gene panel analysis of a heterogeneous group of PMG cases was uninformative for 79.7% patients. The causes for the undiagnosed group may include non-genetic factors, brain specific somatic mutations or variants in genes not yet discovered or known to be associated with brain

malformations, the latter of which may be detected using a broader genetic test such as whole exome or genome sequencing. Seven of the eight patients born of a twin pregnancy did not have a genetic cause identified, consistent with previous studies that have identified twinning as a risk factor for PMG with the causative mechanism thought to be vascular disruption (Curry *et al.*, 2005).

### Monogenic causes

Causative or candidate variants were identified in 13 different genes (Table 1) and included autosomal recessive variants, inherited autosomal dominant variants and *de novo* autosomal dominant variants, non-mosaic and mosaic. Despite the predominance of males affected and 16 X-linked genes tested by one or both gene panels, no X-linked causes were identified. The majority of genetic causes in this cohort of 123 patients implicated tubulin genes (31%) or genes in the mTOR-PI3K-AKT pathway (27%). The significant contribution of tubulin-related mutations to malformations of cortical development has been recognized in previous studies. Heterogeneous cohorts of patients with malformations of cortical development screened for tubulin mutations have yielded a diagnosis in up to 13.3%. (Bahi-Buisson *et al.*, 2014). Seven of the 26 diagnoses (27%) were in mTOR-PI3K-AKT pathway-related genes, *PIK3R2* and *PIK3CA*. The recurrent *PIK3R2* variant p. Gly373Arg constituted 20% of the molecular diagnoses in the entire cohort. Previous studies have identified this variant at similar frequencies, in 10 of 40 (25%) patients with Megalencephaly-PMG-Polydactyly-Hydrocephalus (Riviere *et al.*, 2012) and 19/127 (15%) patients with isolated BPP or Megalencephaly-PMG-Polydactyly-Hydrocephalus (Mirzaa *et al.*, 2015). Beyond these two groups of genes, the causes identified were varied and included isolated and syndromic causes for PMG. Four of the implicated genes are not PMG-associated as published in the GeneReviews Polymicrogyria Overview update in 2018: *ACTG1*, *COL4A1*, *COL4A2* and *FH* (Table 1). These four genes had been included in both panels due to a speculative association with PMG or association with a brain malformation other than PMG and these findings highlight the value of broad genetic testing beyond panels that include only the currently known genetic causes.

### Novel potential chromosomal causes

Two patients included in the study have chromosome copy number variants of uncertain significance that may be relevant to their PMG. One patient with BPP and nephronophthisis has a homozygous deletion of *NPHP1*, a known cause for nephronophthisis but never before reported in association with PMG. As PMG is observed in at least one other ciliopathy, that associated with *AHI1*, it is plausible that the homozygous *NPHP1*

deletion in this patient explains both their brain and renal phenotype, though there are no other published examples of this. There is one published case of cortical malformation identified in a foetus with compound heterozygous variants in *NPHP1*, a pathogenic deletion and a missense variant of unknown significance (Reches et al., 2018). The second case with a possible novel chromosomal cause for their PMG is a male with BPP and a maternally inherited duplication at Xq28 involving *RAB39B*. Duplications of this region, and *RAB39B* specifically, have been associated with intellectual disability but not cortical malformation to date (El-Hattab et al., 2011; Vanmarsenille et al., 2014).

## Genotype–phenotype correlations

Several genotype–phenotype correlations were identified. HC was the most informative clinical feature regarding the likelihood of identifying a genetic cause. A genetic diagnosis was confirmed in 58.3% of patients with macrocephaly and 30.8% in the patients with microcephaly. All patients identified as having a causative or candidate variant in a tubulin gene had a bilateral distribution of PMG and additional brain malformations that are recognized as features of a tubulinopathy (Mutch et al., 2016). Therefore, the presence of this brain phenotype, which includes agenesis/hypoplasia or the corpus callosum, dysplastic basal ganglia, brain stem hypoplasia and hypoplasia/dysplasia of the cerebellum/vermis is highly predictive of a causative variant in a tubulin gene. A causative homozygous variant in *GPSM2* was identified in a patient with the typical PMG distribution and syndromic features of Chudley–McCullough syndrome, which is an important genotype to consider in the prenatal setting as the neurodevelopmental outcome is typically good despite extensive CNS malformation. A likely pathogenic variant in *ACTG1* was identified in a patient with non-syndromic BPP, expanding the spectrum of cortical malformation associated with this gene which has previously been limited to pachygyria. This finding has informed the need to monitor this patient for comorbidities associated with the Baraitser–Winter Syndrome.

## Use of a gene panel

The strength of a gene panel is in its coverage of genes and sensitivity to low-level mosaic variants when compared to exome sequencing. In this study, we detected five causative mosaic variants, three of which were detected with an AF less than 0.15 in blood, a level that would not reliably be detected by clinical exome sequencing. A limitation to the use of a gene panel is that the genomic analysis is limited to the genes included in the panel at the time of its design. This technology prevents future analysis of newly discovered genes and genes not known to be associated with brain development at the time of panel design. Additionally, syndromic causes of

PMG may be missed if the causative gene is not typically associated with PMG. In this case, a clinical assessment of the patient's phenotype and family history may be helpful in determining whether a gene panel or exome sequencing is the more useful test, though access to these tests is also dependent on local availability and resources. A gene panel may be useful to investigate a specific phenotype with suspected mosaic cause. Outside of this, a two-step approach to genome-wide testing is recommended with targeted analysis of exome data focused on genes known to cause PMG and, if negative, an expanded analysis of the clinical exome.

The current rate of gene discovery is high, predominantly due to the application of next-generation sequencing to gene discovery projects. Since the PMG Overview in GeneReviews was updated in 2018 and the panels for these two cohorts were designed, two genes with significant clinical relevance for PMG have been identified. Heterozygous pathogenic missense variants in *SCN3A* were identified by Smith et al. (2018) in 2018 in six unrelated families with speech and oral motor dysfunction and PMG and Chatron et al. (2019) reported four cases from two unrelated families with a lethal PMG syndrome caused by homozygous *ATP1A2* truncating variants in families with a history of migraine in carrier parents. The rate of new gene discovery and the expanding number of molecular pathways implicated in cortical development suggests that diagnoses may have been missed by the gene panel analysis of this cohort as this technology limits the analysis to those genes included in the panel at the time of its design.

## Clinical contribution to genomic analysis

A strength of this study is the clinical contribution to patient phenotyping and variant interpretation. This was made possible by a close working relationship between the clinicians and the testing laboratory in both centres. The cohort was refined to those with potential monogenic cause and this was achieved through clinical interpretation of clinical features, family history and chromosomal variants as being contributory or non-contributory. An example of the clinical contribution to variant prioritization included the re-classification of the PMG in tubulin-related dysgyria and subsequent prioritization of tubulinopathy-related genes. Where there was a strong phenotype match with a gene-disease association identified, sufficient to strengthen the evidence for pathogenicity, this led to re-classification of a variant of uncertain significance in some cases to likely pathogenic. The genotype–phenotype correlations identified in this study can assist the clinician in prioritizing candidate genes and variants for curation. These correlations also help to identify patients who are more likely to benefit from genomic testing thereby optimizing the use of resources, especially in resource-poor settings.

## Conclusions

Genetic analysis of 123 patients with PMG using deep sequencing panels identified a cause in 20.3%. Genetic testing has the highest diagnostic yield in patients with PMG and abnormal head growth. The highest yield is in those with macrocephaly, in which a mosaic variant may be implicated, and deep sequencing should be considered in these cases. The genetic causes for PMG with microcephaly are unlikely to be mosaic and include recessive and *de novo* dominant causes. Where non-mosaic causes are being considered, whole exome or genome sequencing is recommended in preference to a gene panel due to the genetic heterogeneity of PMG and the ability to reanalyse the data as new genes are discovered. The causes for the undiagnosed group may include non-genetic factors, brain-specific somatic mutations or variants in genes not yet discovered or known to be associated with brain malformation, the latter of which may be detected by whole-exome or genome sequencing.

## Supplementary material

Supplementary material is available at *Brain Communications* online.

## Acknowledgements

We thank the patients, their families and their referring clinicians for their participation in this study.

## Funding

C.A.S. was supported by the Flora Suttie Neurogenetics Fellowship funded by the Suttie family and their supporters, the Thyne Reid Foundation and the Macquarie Foundation, and National Health and Medical Research Council Postgraduate Scholarship (APP1133266). The research at the Murdoch Children's Research Institute was supported by the Campbell Edwards Trust, the Genet Foundation and the Murdoch Children's Research Institute Translation Research Fund. P.J.L. was supported by the Vincent Chiodo Foundation. A.J. was supported by a Senior Clinical Investigator Fellowship from the Research Foundation - Flanders. R.J.L. was supported by a Melbourne Children's Clinician Scientist Fellowship.

## Competing interests

The authors report no competing interests.

## References

- Bahi-Buisson N, Poirier K, Fourniol F, Saillour Y, Valence S, Lebrun N, et al.; LIS-Tubulinopathies Consortium. The wide spectrum of tubulinopathies: what are the key features for the diagnosis? *Brain* 2014; 137: 1676–700.
- Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. A developmental and genetic classification for malformations of cortical development: update 2012. *Brain* 2012; 135: 1348–69.
- Chatron N, Cabet S, Alix E, Buenerd A, Cox P, Guibaud L, et al. A novel lethal recognizable polymicrogyric syndrome caused by ATP1A2 homozygous truncating variants. *Brain* 2019; 142: 3367–74.
- Crome L, France NE. Microgyria and cytomegalic inclusion disease in infancy. *J Clin Pathol* 1959; 12: 427–34.
- Curry CJ, Lammer EJ, Nelson V, Shaw GM. Schizencephaly: heterogeneous etiologies in a population of 4 million California births. *Am J Med Genet A* 2005; 137A: 181–9.
- Dixon-Salazar T, Silhavy JL, Marsh SE, Louie CM, Scott LC, Gururaj A, et al. Mutations in the AHI1 gene, encoding joubertin, cause Joubert syndrome with cortical polymicrogyria. *Am J Hum Genet* 2004; 75: 979–87.
- El-Hattab AW, Fang P, Jin W, Hughes JR, Gibson JB, Patel GS, et al. Int22h-1/int22h-2-mediated Xq28 rearrangements: intellectual disability associated with duplications and in utero male lethality with deletions. *J Med Genet* 2011; 48: 840–50.
- Friede R. *Developmental neuropathology*. Berlin: Springer; 1989.
- Jansen A, Andermann E. Genetics of the polymicrogyria syndromes. *J Med Genet* 2005; 42: 369–78.
- Jansen AC, Robitaille Y, Honavar M, Mullatti N, Leventer RJ, Andermann E, et al. The histopathology of polymicrogyria: a series of 71 brain autopsy studies. *Dev Med Child Neurol* 2016; 58: 39–48.
- Kerrigan JF, Aleck KA, Tarby TJ, Bird CR, Heidenreich RA. Fumaric aciduria: clinical and imaging features. *Ann Neurol* 2000; 47: 583–8.
- Lee WS, Stephenson SE, Pope K, Gillies G, Maixner W, Macdonald-Laurs E, et al. Genetic characterisation identifies bottom-of-sulcus dysplasia as an mTORopathy. *Neurology* 2020; 95: e2542–e2551.
- Lee WS, Stephenson SEM, Howell KB, Pope K, Gillies G, Wray A, et al. Second-hit DEPDC5 mutation is limited to dysmorphic neurons in cortical dysplasia type IIA. *Ann Clin Transl Neurol* 2019; 6: 1338–44.
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T; Exome Aggregation Consortium, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; 536: 285–91.
- Leventer RJ, Jansen A, Pilz DT, Stoodley N, Marini C, Dubeau F, et al. Clinical and imaging heterogeneity of polymicrogyria: a study of 328 patients. *Brain* 2010; 133: 1415–27.
- Leventer RJ, Phelan EM, Coleman LT, Kean MJ, Jackson GD, Harvey AS. Clinical and imaging features of cortical malformations in childhood. *Neurology* 1999; 53: 715–22.
- Levine DN, Fisher MA, Caviness VS Jr. Porencephaly with microgyria: a pathologic study. *Acta Neuropathol* 1974; 29: 99–113.
- Mirzaa GM, Conti V, Timms AE, Smyser CD, Ahmed S, Carter M, et al. Characterisation of mutations of the phosphoinositide-3-kinase regulatory subunit, PIK3R2, in perisylvian polymicrogyria: a next-generation sequencing study. *Lancet Neurol* 2015.
- Mutch CA, Poduri A, Sahin M, Barry B, Walsh CA, Barkovich AJ. Disorders of Microtubule Function in Neurons: Imaging Correlates. *AJNR Am J Neuroradiol* 2016; 37: 528–35.
- Oegema R, Cushion TD, Phelps IG, Chung SK, Dempsey JC, Collins S, et al. Recognizable cerebellar dysplasia associated with mutations in multiple tubulin genes. *Hum Mol Genet* 2015; 24: 5313–25.
- Reches A, Hirsch L, Simchoni S, Barel D, Greenberg R, Ben Sira L, et al. Whole-exome sequencing in fetuses with central nervous system abnormalities. *J Perinatol* 2018; 38: 1301–8.

- Riviere JB, Mirzaa GM, O’Roak BJ, Beddaoui M, Alcantara D, Conway RL, et al.; Finding of Rare Disease Genes (FORGE) Canada Consortium. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nat Genet* 2012; 44: 934–40.
- Sadedin SP, Dashnow H, James PA, Bahlo M, Bauer DC, Lonie A, et al.; Melbourne Genomics Health Alliance. Cpipe: a shared variant detection pipeline designed for diagnostic settings. *Genome Med* 2015; 7: 68.
- Smith RS, Kenny CJ, Ganesh V, Jang A, Borges-Monroy R, Partlow JN, et al. Sodium channel SCN3A (Nav1.3) regulation of human cerebral cortical folding and oral motor development. *Neuron* 2018; 99: 905–13 e7.
- Stouffs K, Verloo P, Brock S, Regal L, Beysen D, Ceulemans B, et al. Recurrent NEDD4L variant in periventricular nodular heterotopia, polymicrogyria and syndactyly. *Front Genet* 2020; 11: 26.
- Stutterd CA, Dobyns, WB, Jansen, AMirzaa, G Leventer, RJ, Polymicrogyria overview. In: MP Adam, HH Ardinger, RA Pagon, SE Wallace, LJH Bean, K Stephens, et al. editors. *GeneReviews* (R). Updated 2018 Aug 16 ed. Seattle (WA); 2018.
- Stutterd CA, Leventer RJ. Polymicrogyria: a common and heterogeneous malformation of cortical development. *Am J Med Genet* 2014; 166: 227–39.
- Utsch B, Sayer JA, Attanasio M, Pereira RR, Eccles M, Hennies HC, et al. Identification of the first AHI1 gene mutations in nephronophthisis-associated Joubert syndrome. *Pediatr Nephrol* 2006; 21: 32–5.
- Vanmarsenille L, Giannandrea M, Fieremans N, Verbeeck J, Belet S, Raynaud M, et al. Increased dosage of RAB39B affects neuronal development and could explain the cognitive impairment in male patients with distal Xq28 copy number gains. *Hum Mutat* 2014; 35: 377–83.