



Polymicrogyria is Associated With Pathogenic Variants in *PTEN*

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Objective: Congenital structural brain malformations have been described in patients with pathogenic phosphatase and tensin homologue (*PTEN*) variants, but the frequency of cortical malformations in patients with *PTEN* variants and their impact on clinical phenotype are not well understood. Our goal was to systematically characterize brain malformations in patients with *PTEN* variants and assess the relevance of their brain malformations to clinical presentation.

Methods: We systematically searched a local radiology database for patients with *PTEN* variants who had available brain magnetic resonance imaging (MRI). The MRI scans were reviewed systematically for cortical abnormalities. We reviewed electroencephalogram (EEG) data and evaluated the electronic medical record for evidence of epilepsy and developmental delay.

Results: In total, we identified 22 patients with *PTEN* pathogenic variants for which brain MRIs were available (age range 0.4–17 years). Twelve among these 22 patients (54%) had polymicrogyria (PMG). Variants associated with PMG or atypical gyration encoded regions of the phosphatase or C2 domains of *PTEN*. Interestingly, epilepsy was present in only 2 of the 12 patients with PMG. We found a trend toward higher rates of global developmental delay (GDD), intellectual disability (ID), and motor delay in individuals with cortical abnormalities, although cohort size limited statistical significance.

Interpretation: Malformations of cortical development, PMG in particular, represent an under-recognized phenotype associated with *PTEN* pathogenic variants and may have an association with cognitive and motor delays. Epilepsy was infrequent compared to the previously reported high risk of epilepsy in patients with PMG.

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Phosphatase and tensin homologue (*PTEN*) pathogenic variants have been identified in association with several clinical syndromes that are distinct yet have overlapping features of aberrant growth leading to macrocephaly or macrosomia and a susceptibility to tumor formation.

Examples include Bannayan–Riley–Ruvalcaba (BRR) syndrome (Online Mendelian Inheritance in Man [OMIM] 153480), characterized by macrocephaly, developmental delay, vascular malformations (including hemangiomas), and hyperpigmented macules of the glans penis¹; Cowden

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syndrome (OMIM 158350), characterized by macrocephaly, hamartomas, and increased risk of breast, thyroid, and endometrial cancer; and macrocephaly/autism syndrome (OMIM 605309), characterized by macrocephaly, abnormal facial features, and delayed psychomotor development.² There is clinical overlap among these syndromes, which fall under the general classification of PTEN Hamartoma Syndrome (PTHS).

Patients with *PTEN* variants have been shown to harbor specific brain imaging characteristics reflecting abnormalities in cortical development. Prior studies have noted enlarged perivascular spaces and periventricular white matter abnormalities.^{3,4} A separate study evaluating characteristics of *PTEN*-associated disorders reported that 2 of 14 patients undergoing magnetic resonance imaging (MRI) had Chiari type I malformation but no other associated structural abnormalities, although more than half had associated systemic vascular anomalies.⁵ A more recent study used quantitative evaluation of brain MRI in individuals with PTHS to demonstrate the frequent finding of megacortex callosum, present in 9 of 12 patients (75%) and the malformation of cortical development polymicrogyria (PMG) in 4 of 12 patients (33%).⁶ Malformations, such as PMG, have clinical implications, such as epilepsy, that have not been explored specifically in the context of *PTEN* germline variants.

PMG is a characteristic brain folding pattern that has been associated with numerous genetic alterations as well as nongenetic or acquired causes. Genes that encode proteins of the phosphoinositide-3-kinase (PI3K) pathway, such as *AKT3* and *PIK3CA*, have been implicated.^{7–9} PMG has also been associated with disorders linked to tubulin genes, transcriptional regulators, and numerous other genetic causes of abnormal neuronal migration.⁷ Clinically, seizures have been reported in up to 78% of patients with PMG of any cause.⁷

PTEN encodes a tumor suppressor on chromosome 10q23.31, which catalyzes the degradation of phosphatidylinositol-(3,4,5)-triphosphate generated by PI3K, inhibiting downstream activation of PI3K pathway targets. When *PTEN* is mutated, this PI3K inhibition is reduced, leading to downstream activation of cellular growth pathways, angiogenesis, and cell differentiation.¹⁰ Despite the known regulation of PI3K by PTEN and the known role of PI3K in PMG, there have been no systematic studies of the association between *PTEN* variants and PMG or other cortical abnormalities.

We performed a systematic review of 22 individuals who harbor variants in *PTEN* at a single large pediatric center. We report the genetic characterization, standardized review of MRI characteristics, review of electroencephalography (EEG), and neurodevelopmental characteristics of this cohort. We specifically assessed the rates of brain malformations, and, in those with brain

malformations, the presence of epilepsy and developmental disabilities.

Methods

This study received prior approval by the Boston Children's Hospital (BCH) Institutional Review Board.

Patient Ascertainment

We queried the BCH Radiology database (Nuance mPower, Nuance Communications) for dictations including the term "PTEN." Each identified case was manually reviewed for the availability of a brain MRI and genetic confirmation of a *PTEN* variant. Individuals for whom genetic results could not be confirmed were removed from the study. Five individuals (3 who overlapped with radiological ascertainment) were identified through the BCH Brain Development and Genetics (BrDG) Clinic: patient numbers 2, 3, 4, 10, and 12. A total of 22 individuals with *PTEN* variants were included in this study.

MRI Evaluation

Brain MRIs were systematically reviewed for the presence of PMG by an experienced, board-certified pediatric neuroradiologist (E.Y.). We annotated individuals based upon their MRI characteristics as those having PMG (Patients 1–12) versus those without PMG, or with atypical gyration (Patients 13–22). Cases with increased gyral frequency and distortion of surface anatomy were scored as positive for polymicrogyria if these findings were observed in multiple planes, and as atypical gyration if suggestive of PMG in only one imaging plane. PMG was further classified by location (frontal [F], parietal [P], and perisylvian [Ps]). Other malformations of cortical malformation and other structural abnormalities were also noted if present (eg, callosal dysgenesis, developmental venous anomalies, and white matter changes). Motion-free studies with definition of the cortical ribbon comparable to our best quality studies (usually 3 T MRIs) were designated high quality studies; studies that had deficiencies in resolution, motion, or signal to noise that degraded diagnostic confidence were designated as lower quality.

Clinical Presentation

Developmental history, epilepsy history, and physical examination were assessed directly during clinical encounters for the 5 patients seen by study authors. For the other patients, assessment was based on chart review. In particular, developmental assessments were limited to descriptions provided by review in developmental medicine, genetics, or neurology clinics. EEG or sleep study data were reviewed by a trained epileptologist (C.M.A.) for all individuals who had undergone these studies. To assess intellectual disability and/or global developmental delay

(ID/GDD), chart data was reviewed, and interpreted by a neurodevelopmental specialist. ID was defined as full scale IQ score < 70. For individuals for whom objective neuropsychological data were not available, a developmental quotient was estimated for clinical determination of ID/GDD.

Classification of PTEN Variants

PTEN variants were classified regarding pathogenicity based on consensus recommendations of the American College of Medical Genetics and Genomics (ACMG).¹¹ References used for classification are detailed in Supplementary Table S1.

Targeted Sequencing

Targeted sequencing was undertaken for 3 individuals. Gene capture was performed with molecular inversion probes (MIPs) spanning across exomes of 41 genes previously associated with PMG (see Supplementary Table S2 for a full list of genes). For the MIP design, custom scripts incorporating the MipGen¹ tool were used for dense tiling of > 98% of all targeted bases with an average of at least 2 unique MIPs. The MIP pool was amplified with low cycles (17 cycles), high-fidelity polymerase, and custom common primers. Sequencing libraries were generated by hybridization of MIPs with 250 ng of DNA for 24 hours. Hybridized MIPs were then filled in and ligated, and linear DNA was removed. Captured products were amplified using 15 cycles of polymerase chain reaction (PCR) with custom 8 nt indexing primers and sequenced on the Illumina HiSeq platform with 2 × 150 bp paired end reads. The paired end reads were mapped to the hg19 human genome build using default settings in BWA-mem. All mapped BAMs were processed for germline mutations using default settings within GATK 3.7 Haplotype caller. The resulting joint-called VCF file was annotated using custom Annovar scripts and databases for population allele frequency filtration and missense prediction databases.

Exome Sequencing

For 2 individuals, whole exome sequencing (WES) and data processing were performed by the Genomics Platform at the Broad Institute of the Massachusetts Institute of Technology (MIT) and Harvard. Libraries from DNA samples (> 250 ng of DNA, at > 2 ng/μL) were created with an Illumina Nextera or Twist exome capture (~38 Mb target) and sequenced (150 bp paired reads) to cover > 80% of targets at 20× and a mean target coverage of > 100×. Sample identity quality assurance checks were performed on each sample. The exome sequencing data was de-multiplexed and each sample's sequence data were

aggregated into a single Picard BAM file. Exome sequencing data was processed through a pipeline based on Picard using the base quality score recalibration and local realignment at known indels. The BWA aligner was used for mapping reads to the human genome build 38. Single nucleotide variants (SNVs) and insertions/deletions (indels) were jointly called across all samples using Genome Analysis Toolkit (GATK) HaplotypeCaller package version 3.5. Default filters were applied to the SNV and indel calls using the GATK Variant Quality Score Recalibration (VQSR) approach. Annotation was performed using Variant Effect Predictor (VEP). Last, the variant call set was uploaded for collaborative analysis between the Broad Institute Center for Mendelian Genomics and investigator. Similar variant calling approaches were used in prior Broad Institute publications.¹²

Results

Patient Cohort and Genetics

In total, between ascertainment from the BCH Radiology database and direct referral to our BrDG Clinic, we identified a total of 22 patients harboring *PTEN* variants (Table 1; Fig 1A). By searching MRI requisition forms or radiologist dictation for the search term "*PTEN*," we identified a total of 31 individuals out of 60,086 MRIs in the database, of whom 20 individuals had confirmed *PTEN* variants based on clinical sequencing data or clinical documentation. In addition, 5 clinically ascertained individuals with *PTEN* variants and brain malformations were included in the study; 3 of these 5 individuals had also been identified by the Radiology database search. Of the total 22 individuals that comprise our *PTEN* cohort, 14 (63.6%) were boys, 8 (36.4%) were girls, and ages ranged from 2.8 years to 17 years.

We annotated individuals as those having PMG (Patients 1–12) and those without PMG (Patients 13–22). All 22 ascertained patients harbored heterozygous *PTEN* variants. Patient 20 harbors an additional benign *PTEN* variant in the 5'UTR in addition to the pathogenic variant. Fourteen variants were SNVs that altered the coding sequence or splice sites, 7 variants were small indels, and 1 patient (Patient 22) harbored a small chromosomal deletion that included the *PTEN* gene. All individuals harbored pathogenic or likely pathogenic variants by ACMG criteria,¹¹ except for Patient 2, Patient 8, and Patient 13 who harbored variants of uncertain significance (VUS). Despite classification as VUS, these individuals exhibited features consistent with PTHS on review of clinical symptoms, and their associated variants were not observed in control population databases^{13,14} (Supplementary Table S1). Thus, these individuals were included in our

TABLE 1. Complete List of Patients Included in This Study and Salient Genetic and MRI Findings

Patient	Age at evaluation	Sex	OFC SD (age yr)	Coding variant	Predicted functional change	ACMG classification	MRI finding
1	17 yr	M	+6.9 (17)	c.209 + 5 G > A	Splice site	Pathogenic	F Ps PMG
2	14 yr	M	+4.6 (14)	c.380G > C	p.Gly127Ala	VUS	F P PMG
3	2.8 yr	M	+5.6 (2.8)	c.388C > T	p.Arg130Ter	pathogenic	F Ps PMG
4	4.75 yr	F	+5.0 (3.75)	c.388C > T	p.Arg130Ter	Pathogenic	DMEG (+PMG)
5	12.5 yr	M	+6.4 (12)	c.389G > A ^a	p.Arg130Gln	Pathogenic	F Ps PMG
6	8 yr	F	4.3 (8)	c.406 T > C	p.Cys136Arg	Pathogenic	PMG (vs. FCD)
7	0.4 yr	M	n.d.	c.464A > C	p.Tyr155Ser	Pathogenic	F P Ps PMG
8	6 yr	F	+6.4 (6)	c.521A > G	p.Tyr174Cys	VUS	F P PMG
9	8 yr	M	+4.1 (3.6)	c.611delC	p.Pro204Glnfs*17	Likely Pathogenic	F Ps PMG
10	16 yr	M	+6.9 (16)	c.737C > T	p.Pro246Leu	Pathogenic	F P Ps PMG
11	16 yr	M	+6.6 (16)	c.955insA	p.Thr319Asnfs*6	Pathogenic	F P Ps PMG
12	16 yr	M	+7.0 (6)	c.1027delG	p.Val343Ter	Likely pathogenic	F P Ps PMG
13	21 yr	M	+5.5 (19)	c.-1034-1030dupGCCCT	Promotor	VUS	No cortical abnormality
14	11 yr	F	+4.7 (10)	c.27delT	p.Ser10Alafs*14	Pathogenic	No cortical abnormality
15	12 yr	F	+9.0 (11)	c.164 + 1G > A	Splice Site	Pathogenic	Atypical gyration
16	9 yr	M	+5.4 (8)	c.323 T > C*	p.Leu108Pro	Pathogenic	Atypical gyration
17	13 yr	F	+2.9 (10)	c.686C > A	p.Ser229Ter	Pathogenic	No cortical abnormality
18	5 yr	F	+4.5 (5)	c.737C > T	p.Pro246Leu	Pathogenic	SEGMH
19	8 yr	M	+4.8 (6)	c.1027-1G > A	Splice site	Pathogenic	No cortical abnormality
20	24 yr	F	+3.8 (20)	c.1110-1111ins ATAGT	p.Asp371Ilefs*47	Likely Pathogenic	No cortical abnormality
21	14 yr	M	n.d.	c.1176delT	p.Phe392Leufs*24	Likely Pathogenic	No cortical abnormality
22	11 yr	M	+4.7 (11)	deletion chr10q23	Deletion	Pathogenic	Atypical gyration

^acDNA variant inferred from amino acid change documented in the electronic medical record.

ACMG = American College of Medical Genetics and Genomics; DMEG = dysplastic megalencephaly; F = frontal; FCD = focal cortical dysplasia; MRI = magnetic resonance imaging; n.d. = data not available; OFC = occipitofrontal circumference; P = parietal; PMG = polymicrogyria; Ps = perisylvian; SEGMH = subependymal grey matter heterotopia; VUS = variants of uncertain significance.

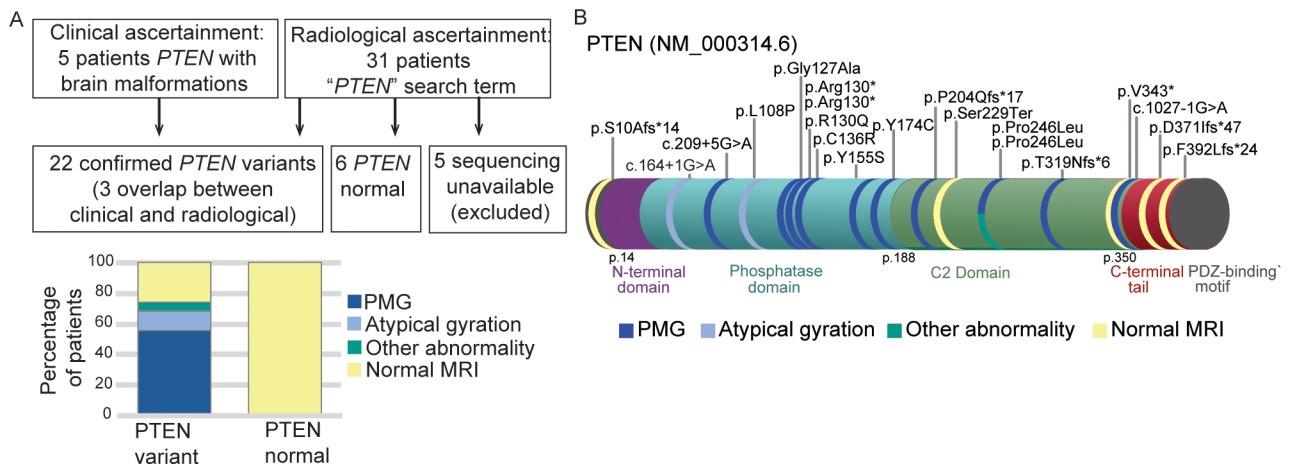


FIGURE 1: PTEN patient cohort. (A) Schematic of patient ascertainment and association with MRI characteristics. (B) Mutational spectrum of PTEN associated with cortical abnormalities. Promoter region variant and chromosomal deletion including entirety of PTEN are not represented. MRI = magnetic resonance imaging; PMG = polymicrogyria; PTEN = phosphatase and tensin homologue.

study based on our determination that these individuals' disorders were likely related to *PTEN*.

Due to the known incidence of multiple molecular diagnoses,¹⁵ particularly in patients that have autosomal dominant variants, we performed additional DNA sequencing in 4 patients who could be consented for additional sequencing and ruled out additional gene variants that may be contributing to brain malformations. Targeted sequencing was performed for 41 genes typically associated with PMG in Patients 3, 4, and 10 (Supplementary - Table S2). In addition, WES was performed on Patient 4 and Patient 10. No additional variants other than those in *PTEN* were identified that were likely to represent a cause of polymicrogyria for these individuals.

Retrospective MRI Review Reveals Frequent Cortical Abnormalities in Patients With PTEN Pathogenic Variants

Standardized review of radiologic features of all 22 patients with *PTEN* variants in our cohort revealed a significant association of *PTEN* pathogenic variants with the radiographic appearance of PMG on MRI in comparison to 6 individuals for whom *PTEN* sequencing was normal (see Fig 1; Table 1; $p = 0.002$, Fisher's exact test). The individuals with normal *PTEN* status were also identified through radiological database query for search term "*PTEN*," likely due to initial clinical concern for PTHS, and thus their lack of PMG despite similar method of ascertainment indicates that it is less likely that findings of PMG in our *PTEN* cohort are simply due to radiological ascertainment bias. Just over half (12/22, 54%) of the individuals we assessed with *PTEN* variants had PMG on MRI, and an additional 13.6% (3/22) showed atypical gyral patterns that were suggestive of PMG but that could

not be confirmed in multiple radiographic planes. We classified Patient 4 as having PMG, although we note that this patient's MRI would best be described as dysplastic megalencephaly (DMEG) due to the appearance of overgrowth, transmantle/subcortical grey matter heterotopia, and cortical dysplasia, in addition to radiologic appearance of polymicrogyria (Supplementary Table S3).

Among the 12 individuals with *PTEN* variants and PMG (Patients 1–12), the pattern of PMG involved the frontal and parietal convexities predominantly at the depth of the sulci, for example, inferior and superior frontal sulci (Fig 2A, B). In addition, 8 individuals had PMG involving the perisylvian regions, and when present, the perisylvian region was the most conspicuous site of involvement. Some areas of PMG were subtle, resulting in fine areas of increased gyration and only localized disturbance of surface anatomy (Fig 2C). In 3 individuals classified as having an atypical gyral pattern (Fig 2D), abnormal folding could not be convincingly demonstrated in multiple planes as required for PMG according to our classification. The cortical areas exhibiting atypical gyration were also perisylvian (1 patient) or frontal (2 patients).

PTEN variants associated with brain abnormalities tended to encode regions of the *PTEN* phosphatase domain (Fig 1B). Of the 10 individuals who harbored variants in the phosphatase domain, 8 had PMG (including Patient 4 with PMG/DMEG) and 2 had an atypical gyral pattern. In comparison, of the 7 individuals with variants in the *PTEN* C2 domain, 4 had PMG, 1 had subependymal grey matter heterotopia (SEGMH), and 2 had no brain malformations. None of the 5 remaining variants, which were located in the promoter region, N-terminal or C-terminal domains, or gene deletion, were associated with brain abnormalities.

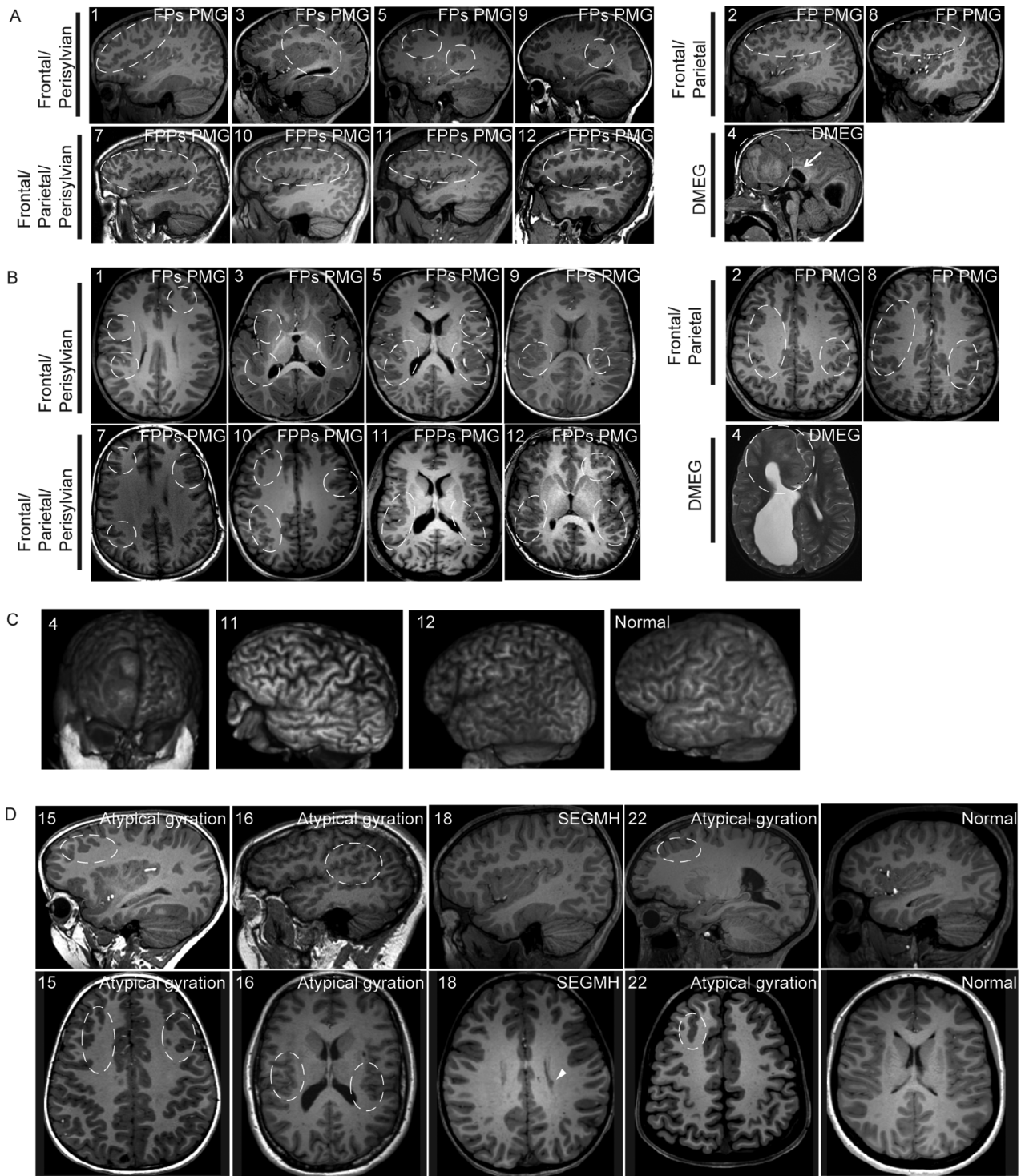


FIGURE 2: Cortical abnormalities associated with PTEN variants. Sagittal (A) and axial (B) views of affected individuals with structural brain abnormalities. (C) 3D reconstructed images of selected cortical abnormalities. (D) Sagittal and axial MRI images from individuals with atypical gyration (appearance of PMG observed only in 1 radiological plane) or with SEGMH. Upper left, patient identifier. Dotted regions highlight affected brain areas. DMEG = dysplastic megalencephaly; MRI = magnetic resonance imaging; PMG = polymicrogyria; PTEN = phosphatase and tensin homologue; SEGMH = subependymal grey matter heterotopia.

The quality of the MRI study performed affected radiologic determination of PMG. Nine of the 12 studies which were called as PMG were high-quality studies, performed using 3 T MRI without significant motion

degradation or artifact. In contrast, only 2 of the 8 studies that we reported as showing atypical gyral patterns or no abnormalities were of high quality. Only 6 of 12 patients with PMG were noted to have PMG on the initial clinical MRI

report, whereas only 1 case that was initially dictated as having PMG was reclassified as atypical gyration in our study.

The full table of MRI findings in each patient can be found in Supplementary Table S3. Except for Patient 4 who had dysplastic megalencephaly and subcortical heterotopia, none of the other individuals with polymicrogyria had heterotopia or hamartoma. Patient 18, who did not have polymicrogyria, had a single focus of SEGMH. Additional imaging findings in the *PTEN* cohort included white matter abnormalities and vascular abnormalities. Vascular abnormalities included a scalp venous malformation in one individual, an arteriovenous malformation and a sphenoid wing venous malformation. All patients with *PTEN* variants had macrocephaly with head circumference measurements ranging between +2.9 SD up to +9 SD at last measurement. The mean was nominally higher in individuals with PMG (+5.8 SD) compared to those without (+5.0 SD).

Patients With PMG in the Setting of PTEN Variants Do Not Have a High Rate of Epilepsy

Because the presence of PMG has previously been associated with high risk for epilepsy, we reviewed the EEG characteristics and risk of epilepsy in our patient cohort (Table 2). We reviewed clinical records and EEG data to determine whether PMG was associated with epilepsy or electrographic brain abnormalities in this cohort.

Among 12 patients with *PTEN* variants and PMG, 2 individuals (16.7%) had epilepsy. Patient 4 had DMEG

on MRI and multiple reported seizures during infancy, including subclinical seizures and infantile spasms. Diagnosis of epilepsy was determined from chart review as EEG data were not available at our institution. Patient 12 had epilepsy diagnosed at the age of 6, with seizures several times per year on lamotrigine monotherapy, and EEG showed continuous generalized slowing with right posterior and midline spikes on review.

Of the patients who had PMG but did not have epilepsy, we identified 1 patient with abnormal EEG among 3 who had an EEG performed for any reason. Patient 3 had an EEG performed as part of the work-up for developmental delay, which showed intermittent generalized slowing and midline spikes. Patient 1 and Patient 10 had normal EEGs.

Of the 10 individuals with *PTEN* variants without PMG, none had documented seizures based on chart review. Four patients had EEGs, and 2 had limited EEG data from sleep studies (see Table 2). Patient 15 had an abnormal EEG with sleep-activated right and left centrotemporal spikes with tangential dipole. This EEG was performed for evaluation of nocturnal crying and urinary incontinence, and the episodes were ultimately diagnosed as night terrors. The other 4 EEGs (which includes 2 EEGs from sleep studies) were normal. In summary, most EEGs revealed normal background with no evidence of encephalopathy in the group without definite brain abnormality, and spikes were seen only in 1 individual (1 abnormal of 5 available EEGs) with a pattern that is

TABLE 2. Electrographic Findings of Patients with *PTEN* Variants Who Underwent Electroencephalography

Patient	PMG	Reason for EEG / age performed	Results
1	Yes	Done for inattention / 16 yr	Normal
3	Yes	Developmental delay and ASD / 2 yr	Intermittent slowing and midline spikes
10	Yes	Done as a routine / 14 yr	Normal awake and asleep
12	Yes	Events concerning for apparent GTCs / 6 and 12 yr	At 6 yr: continuous generalized slowing, right posterior and midline spikes. At 12 yr: normal awake and asleep EEG
14	No	Staring spells / 13 yr	Normal EEG, events captured, not seizures
15	No	Single nocturnal episode of crying and urinary incontinence / 9 yr	Right and left sleep activated centrotemporal spikes, otherwise normal background
18	No	Developmental delay and ASD / 3 and 4 yr	Normal background with right parietal spikes
19	No	Sleep study	Normal
22	No	Sleep study	Normal

ASD = autism spectrum disorder; EEG = electroencephalogram; GTC = Generalized tonic-clonic seizure; PMG = polymicrogyria; PTEN = phosphatase and tensin homologue.

seen commonly in pediatric epilepsy but was present in this case in a child without clinical epilepsy.

The number of individuals with epilepsy was not significantly different between patients with versus without PMG (p value = 0.48, Fisher's exact test). However, it is notable that epilepsy was generally infrequent in our cohort of individuals harboring *PTEN* variants, including in those patients with PMG.

Neurodevelopmental Differences Associated With Cortical Abnormalities in Patients With *PTEN* Variants

Rates of cognitive disability were found to be higher in the group of patients with PMG, although our small sample size precluded statistical significance (Fig 3A; Table 3). We utilized data from either full-scale IQ, developmental quotient as calculated from formal neuropsychiatric assessment, or neurology clinical assessment. We assessed whether or not ID/GDD were present in each case. We excluded individuals for which a clear determination could not be made based on available data, although the cognitive/developmental data for each case, including the excluded individuals, are noted in Table 3. Five of 9 (55%) individuals with PMG for whom cognitive status could be ascertained were noted to have GDD/ID, compared with 2 of 8 (25%) individuals without PMG. Although this difference is not statistically significant (p -value = 0.33), some of the delays in patients with cortical abnormalities were quite severe, including 1 patient who spoke only 15 words by age 7 years and another who could only communicate with 20 to 25 signs by age 4 years 9 months.

In general, a high rate of motor delay was noted among all individuals with *PTEN* variants, and a nominally higher rate in those with PMG (Fig 3B; Table 3). We determined motor delay based upon clinically documented parental reports of acquiring the ability to walk

independently > 18 months. In summary, 6 of 9 (66.7%) patients with PMG for whom motor developmental data were available were noted to have motor delays. In patients who did not have PMG, 4 of 8 (50%) patients for whom developmental data were available had motor delays. Speech delay was not assessed as there was inconsistent reporting in the electronic medical record, and rates of primary language delay is confounded by rates of cognitive disability.

Finally, we found that rates of autism spectrum disorder (ASD; previously, pervasive developmental disorder [PDD]) were higher in the *PTEN* cohort with PMG. Four of 12 individuals (33.3%) carried a diagnosis of ASD (diagnosed between the ages of 2 and 5 years), with 1 additional individual described as having difficulty with social nuances but not meeting diagnostic criteria for ASD. In comparison, only 1 of 10 individuals (10%) without PMG carried a diagnosis of ASD (Fig 3C).

Discussion

Here, we expand the phenotype of *PTEN*-associated disorders to include a strong association with cortical malformations, in particular PMG, always in the setting of macrocephaly. Additional brain malformations identified include SEGMH and DMEG. We note that individuals with PMG in our cohort also had higher rates of ASD, motor delay, and ID, although larger cohorts are necessary to confirm the strength of this association.

Variants associated with PMG or atypical gyration were frequently located in the phosphatase domain of *PTEN*, suggestive of a genotype–phenotype relationship. Targeted sequencing of additional known genes associated with PMG in 3 individuals did not identify alternative causes of PMG. However, we cannot rule out the possibility of mosaic variants in these individuals or alternative causes in other individuals that did not undergo targeted

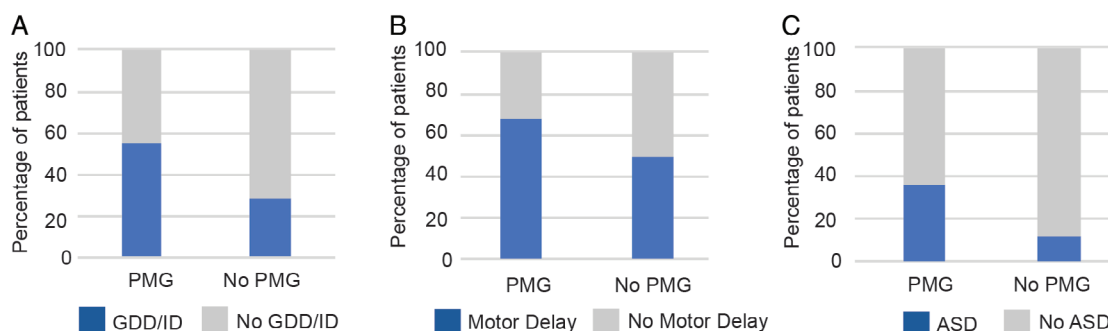


FIGURE 3: Comparison of (A) percentage of individuals with global developmental delay or intellectual disability (GDD/ID), (B) percentage of individuals with delayed motor development, and (C) percentage of individuals with ASD, in individuals with *PTEN* variants with or without polymicrogyria. ASD = autism spectrum disorder; GDD = global developmental delay; ID = intellectual disability; PMG = polymicrogyria; *PTEN* = phosphatase and tensin homologue. [Color figure can be viewed at www.annalsofneurology.org]

TABLE 3. Cognitive, Motor, and Behavioral Development of Patients with *PTEN* Variants

Patient #	ID/GDD	Motor delay	ASD	Assessment method for ID/GDD
1	No	None	No	Full scale IQ 92
2	Yes	Walk at 3–4 yr	No	Full scale IQ 57
3	Yes	Sitting at 18 mo	Yes	Bayley III at 39 mo showed problem solving DQ 36% and language DQ 29%
4	Yes	Sitting at 18 mo	No	20–25 communicative signs at 4 yr 9 mo
5	No	Unknown, Receives OT	Yes	Scales of independent behavior 103
6	No	Walk at 19 mo	No	Age appropriate functioning reported by neurologist
7	Unknown	Unknown	Unknown	n/a
8	Uncertain	Sitting at 10 mo	No	No objective assessment, DQ at least 67% based on review of function reported in neurology clinic visit
9	Yes	Unknown	Yes	Diagnosis in electronic medical record
10	No	Walk at 20 mo	No	Full scale IQ 114
11	Uncertain	Sit at 8 mo	No	No objective assessment
12	Yes	Walk at 3 yr	Yes	15 words at age 7 yr, impaired self-help
13	No	Walk at 1.5 yr	No	Age appropriate comprehension on Gray Oral Reading Paragraph 4
14	Yes	Walk at 16 mo	Yes	Mild intellectual disability reported in electronic medical record
15	Uncertain	Walk at 2 yr	No	IEP in school only supports math
16	No	Walk by 15 mo	No	Delayed 1 yr in elementary school
17	No	None	No	No cognitive concerns by clinicians
18	No	Delayed per clinician	No	At 6 yr 9 mo: KABC-II 79, Kauffman EVT-II 79
19	No	Walk at 21 mo	No	Functioning at grade level with 504 plan
20	Unknown	Unknown	Unknown	n/a
21	Uncertain	Walk at 20 mo	No	Separate classroom special education classes
22	Yes	Walk at 3 yr	No	At 9 yr: reported functioning at kindergarten level by special needs school (development quotient 55%)

ASD = autism spectrum disorder; DQ = developmental quotient; EVT-II = Expressive Vocabulary Test Second Edition; GDD = global developmental delay; ID = intellectual disability; IEP = Individualized Education Plan; KABC = Kaufman Assessment Battery for Children 2; n/a = not applicable; OT = occupational therapy; PTEN = phosphatase and tensin homologue.

sequencing. Somatic variation has been indeed established as a cause of focal structural brain abnormalities.¹⁶

Rates of PMG reported in patients with PTHS have varied widely ranging from 0 to 33%.^{5,6,17} In our study, we find a high rate with just over half of our *PTEN* cohort showing MRI evidence of PMG (54.5%). Although ascertainment bias can be a concern due to identification of individuals through a radiology database, we note that the individuals found via this radiology

search who had normal *PTEN* status despite clinically suspected PTHS did not have cortical malformations. Furthermore, only 2 individuals were clinically ascertained that were not identified by radiology search, and even if they were excluded, the rate of PMG would be 50%.

Our data suggest 2 potential explanations for the wide range of reported PMG prevalence in prior *PTEN* studies: the localized high frequency nature of the PMG

(finesse) in patients with *PTEN* and the general requirement for high quality brain imaging (ie, 3 T MRI). Specifically, PMG can be difficult to detect when thick, nonisotropic brain MRI sequences are obtained or when image noise obscures the increased gyral frequency characteristic of this cortical malformation. Therefore, it is logical that higher quality studies are preferred for detection of cortical abnormalities. Consistent with this concept, the prior study, which reported a rate of PMG of 33%, used only 3 T MRI images.⁶ In our institution, patients with macrocephaly and developmental delay are generally scanned at 3 T with a high-density multichannel head coil (32 or 64-channel), high resolution imaging technique, and at least one isotropic sequence. However, several of the patients in our cohort were imaged for other reasons or had lower resolution outside imaging, which was not repeated. We showed that improved scan resolution increased the ability of the radiologist to call it PMG. Therefore, it is possible that our cohort may actually under-represent the true proportion of individuals that have cortical malformations (ie, atypical gyration or normal brain MRIs may be false negatives for PMG).

Forty-one percent of individuals in our cohort exhibit cortical white matter abnormalities, which have been described previously in association with *PTEN*³ (Supplementary Table S3). Although the prevalence of white matter changes in our cohort is lower than the prior report, this difference is likely related to the fact that patients from the cited study were recruited predominantly from patients referred to leukodystrophy centers for unclassified white matter disorders. Interestingly, it has separately been found that individuals with *PTEN* variants and white matter abnormalities may still have normal intelligence, thus clouding the picture of whether the neurodevelopmental phenotype of *PTEN* is related primarily to dysfunction of the white matter.¹⁸

The radiographic findings in our *PTEN* cohort are important for both diagnosis and patient counseling. Developmental delay and macrocephaly are common indications for brain MRIs in children. Our data would suggest that the finding of PMG should prompt consideration of *PTEN* hamartoma syndrome in addition to other syndromes, such as megalencephaly-polydactyly-polymicrogyria-hydrocephalus syndrome (MPPH)/ megalencephaly-capillary malformation (MCAP), a differential that can change genetic testing and can also lead to changes in approach to clinical evaluation (e.g., dermatologic evaluation). Future prospective studies of patients who present with macrocephaly and PMG identified on MRI would be helpful to clarify how many of the individuals fitting this phenotype have pathogenic variants in *PTEN*.

Conversely, the finding of a cortical malformation need not cast into doubt the diagnosis of *PTEN*-associated disorder. In our clinically ascertained cohort, multiple patients were referred to the BrDG clinic due to the noted PMG despite having a known *PTEN* variant. One family was concerned for a progressive brain abnormality given that a prior MRI had been reported to them as normal. Another family was fearful of epilepsy reported to be associated with PMG. This anxiety may be mitigated by the knowledge that these brain abnormalities are in fact a frequent finding associated with *PTEN* variants and that epilepsy is not universal—in fact, it was not common—in patients with *PTEN*-related PMG.

Patients with PMG in our study of patients with *PTEN* variants have a much lower incidence of epilepsy (16%) when compared more generally to reported rates of epilepsy—up to 87% associated with PMG.¹⁹ Furthermore, epilepsy for patients with PMG has been reported to be difficult to control,^{19,20} again in contrast to the 2 patients with epilepsy in our cohort. Reasons for this difference may include ascertainment bias, as the incidence of epilepsy in patients with PMG is likely to be higher in patients seen in epilepsy clinics versus other clinics. Another consideration is the improved detection of PMG given advancements in MRI technology and its increased use in patients with developmental disorders. Bilateral perisylvian PMG (present in most of our patients) is significantly correlated with lower age of seizure onset as well (12 months),²⁰ thus the current ages of individuals in our cohort is not likely the reason for the relatively low epilepsy prevalence in our population.

One intriguing possibility may be that *PTEN* pathogenic variants either modulate this epileptogenic effect or drive a structural pattern of PMG that is distinct from other causes of PMG. Patients who have PMG as part of MCAP and MPPH syndrome, caused by variants in the PI3K, which is regulated by *PTEN*, have been also reported to have a relatively low (38%) incidence of epilepsy,⁹ although when epilepsy is present in this setting it can be very severe and difficult to control. This difference in epilepsy prevalence for patients with PMG may reflect subtle cytoarchitectural differences as radiographically identified PMG has been shown to represent a spectrum of cortical abnormality at a histological level.^{21,22} Regardless of any underlying histologic differences, our findings suggest that risk of seizures and likelihood of developing drug-resistant epilepsy are both lower than might be expected based on imaging findings and analogy to other forms of megalencephalic PMG.

The individuals with cortical abnormalities in our cohort also had higher rates of GDD/ID, motor delay, and ASD, although our cohort size is insufficiently

powered to determine the significance of these developmental findings. Consistent with prior reports in association with *PTEN*-associated syndromes,^{5,23} we note a high incidence of motor and speech delays regardless of the presence of cortical abnormalities. Thus, patients with *PTEN* variants, particularly those presenting with PMG, should be assessed and closely monitored for features of ASD and developmental delay, particularly when cortical abnormalities are present.

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Author Contributions

D.D.S., C.M.A., E.Y., A.P., and C.A.W. contributed to the conception and design of the study. D.D.S., C.M.A., S.S., L.R., R.D., A.Y.C., E.Y., A.P., and C.A.W. contributed to the acquisition and analysis of

data. D.D.S., C.M.A., E.Y., and A.L. contributed to drafting the text and preparing the figures. The Brain Development Genetics Study Group includes the following individuals who had a key role in data collection and evaluation of individual patients included in this study: Mira B. Irons, MD (American Medical Association; Department of Pediatrics, Feinberg School of Medicine, Chicago, Illinois); Ervin L. Johnson 3rd, MD, PhD (Department of Neurology, Boston Children's Hospital); Mayra Martinez Ojeda, MD (Division of Genetics and Genomics, Boston Children's Hospital and Harvard Medical School); Heather E. Olson, MD, MS (Department of Neurology, Boston Children's Hospital and Harvard Medical School); Mustafa Sahin, MD, PhD (Department of Neurology, Boston Children's Hospital and Harvard Medical School); Coral M. Stredny, MD (Department of Neurology, Boston Children's Hospital); and Wen-Hann Tan, MD (Division of Genetics and Genomics, Boston Children's Hospital and Harvard Medical School).

Potential Conflicts of Interest

The authors declared no conflict of interest.

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