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ARTICLE

The ClinGen Brain Malformation Variant Curation Expert Panel: Rules for somatic variants in *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2*

Abbe Lai[1,2,3], Aubrie Soucy[1,3], Christelle Moufawad El Achkar[2,4], Anthony J. Barkovich[5], Yang Cao[6], Marina DiStefano[7,8], Michael Evenson[6], Renzo Guerrini[9], Devon Knight[2], Yi-Shan Lee[6], Heather C. Mefford[10], David T. Miller[1,3], Ghayda Mirzaa[11,12], Ganesh Mochida[1,3], Lance H. Rodan[1,3,4], Mayher Patel[7], Lacey Smith[2], Sara Spencer[13], Christopher A. Walsh[1,3,4,7], Edward Yang[14], Christopher J. Yuskaitis[2,4], Timothy Yu[1,4,7,*], Annapurna Poduri[2,4,7,*]; On behalf of the ClinGen Brain Malformation Variant Curation Expert Panel

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ABSTRACT

Purpose: Postzygotic (somatic) variants in the mTOR pathway genes cause a spectrum of distinct developmental abnormalities. Accurate classification of somatic variants in this group of disorders is crucial for affected individuals and their families.

Methods: The ClinGen Brain Malformation Variant Curation Expert Panel was formed to curate somatic variants associated with developmental brain malformations. We selected the genes *AKT3, MTOR, PIK3CA*, and *PIK3R2* as the first set of genes to provide additional specifications to the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) sequence variant interpretation guidelines, which currently focus solely on germline variants.

Results: A total of 24 of the original 28 ACMG/AMP criteria required modification. Several modifications used could be applied to other genes and disorders in which somatic variants play a role: 1) using variant allele fraction differences as evidence that somatic mutagenesis occurred as a proxy for *de novo* variation, 2) incorporating both somatic and germline evidence, and 3) delineating phenotype on the basis of variable tissue expression.

Conclusion: We have established a framework for rigorous interpretation of somatic mosaic variants, addressing issues unique to somatic variants that will be applicable to many genes and conditions.

Timothy Yu and Annapurna Poduri contributed equally.

*Correspondence and requests for materials should be addressed to Annapurna Poduri, Department of Neurology, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115. *E-mail address:* annapurna.poduri@childrens.harvard.edu OR Timothy Yu, Department of Pediatrics, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115. *E-mail address:* timothy.yu@childrens.harvard.edu

Affiliations are at the end of the document.

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Introduction

Brain malformations comprise a group of developmental central nervous system disorders associated with distinctive radiographic presentations often suggestive of unique genetic etiologies[1] or biological mechanisms.[2] A particularly interesting group of brain malformations are those manifesting as focal cortical dysplasia (FCD), hemimegalencephaly, and polymicrogyria with megalencephaly. Pathogenic (P) variants in multiple genes have been implicated in this group of disorders, many arising from postzygotic events affecting only a subset of tissues in the affected individual. Embryonic tissue arising after such an event exhibits genetic mosaicism, in which the tissue contains a mixture of variant-positive and variant-negative cells. This is an extension of the role of somatic variants beyond the cancer realm into developmental brain disorders. Surgically resected brain tissues from patients with focal epilepsy were evaluated to identify the first evidence of somatic variants in mTOR pathway genes resulting in epileptogenic cortical malformations.[3,4] It quickly became apparent that there is an overlap between the genetic mechanisms involved in cancer and those that cause these developmental lesions. The same variants identified in tumor samples, which were functionally noted to result in a growth advantage for the cells, were also identified in developmental brain lesions.[5] Leveraging this information, several groups have identified postzygotic variants in multiple genes related to the mTOR pathway in a wide spectrum of developmental brain malformations.[6-8]

Many mTOR pathway genes are established oncogenes and have been the subject of extensive research.[9,10] The association of developmental brain lesions in patients with epilepsy with variants in the mTOR pathway makes these disorders potentially treatable with mTOR inhibitors, which are now being used in clinical trials to assess efficacy for seizure control and developmental progress in the face of structural malformations.[11,12]

The Clinical Genome Resource (ClinGen) has created a framework to allow for the establishment of expert panels (EPs) to refine the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) sequence variant interpretation guidelines for specified gene–disease associations.[13] In recognition of the importance of accurate genetic diagnosis for individuals with developmental brain malformations and to provide a generalizable framework for interpreting somatic variants applicable to these and other conditions, we formed the ClinGen Brain Malformation Variant Curation Expert Panel (BMVCEP). In this article, we present the results of our processes to develop a curation framework for variants in 4 representative genes of the mTOR pathway: *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2*.

Materials and Methods

We selected the 4 most common genes associated with brain malformations caused by mTOR pathway gain of function (GOF)—*AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2*—for initial ACMG/AMP guideline specification. In accordance with ClinGen requirements, the BMVCEP worked with the Mondo Disease Ontology to create the term "overgrowth syndrome and/or cerebral malformations due to abnormalities in mTOR pathway genes" (OCMMPG) to group these disorders into a single disease entity for curation. Although this group of disorders may result from either loss-of-function (LOF) or GOF variants in various genes, these 4 genes specifically involve somatic mosaic GOF variants. We devised a framework to interpret these variants, with consideration of several factors not accounted for in the existing ACMG/AMP sequence variant interpretation guidelines, which focus predominantly on germline and LOF variations.[14]

The BMVCEP was assembled through a cooperative agreement with the National Institutes of Health and included multidisciplinary experts in the mosaic brain disorders field: neurologists, neuroradiologists, clinical and molecular geneticists, genetic counselors, bioinformaticians, and research scientists, many with expertise in neuroscience, genetics, and bioinformatic analyses. This EP was affiliated with the Neurodevelopmental Disorders Clinical Domain Working Group and followed the ClinGen Variant Curation Expert Panel approval process (https://clinicalgenome.org/site/assets/files/3635/variant_curation_expert_panel_vcep_protocol_version_9-2_3.pdf). All EP members disclosed conflicts of interest. A comprehensive list of our members is available at https://clinicalgenome.org/affiliation/50020/.

Variants were identified from relevant publications and publicly available databases, including ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/), and the Catalogue Of Somatic Mutations In Cancer (https://cancer.sanger.ac.uk/cosmic) in 2018 and 2019. Relevant publications were extracted from PubMed using the query, "AKT3" OR "MTOR" OR "PIK3CA" OR "PIK3R2" AND "Overgrowth" AND "Brain". Curators evaluated the resulting 53 publications to extract variants, which were used to pilot modifications to the existing ACMG/AMP variant interpretation scoring system.

Our classification efforts began with a detailed review of the variant interpretation rules from the original 2015 ACMG/AMP publication. The original 28 ACMG/AMP criteria were systematically evaluated for potential gene- or disease-specific specifications and modifications relevant to *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2*. This EP worked with several related ClinGen working groups (Sequence Variation Interpretation [SVI], PTEN, Hearing Loss, and Intellectual Disability/Autism) to review specifications and maintain cross-group consistency.

We systematically evaluated 43 variants across the 4 genes. Curations were performed by 2 independent biocurators and discussed at bimonthly review meetings. Conflicting variant interpretations were resolved by deliberation and consensus of the EP, with a quorum of experts required to establish consensus.

Each variant curator used a customized scoring worksheet that we developed (Supplemental Table 1) to interpret variants in a standardized fashion with consistent logic and formatting. After curation, the scoring worksheet populated a paragraph summarizing the criteria met for that variant (Supplemental Table 2), which was then transferred to the ClinGen Variant Curation Interface.

Consensus approval of the amendments was obtained from all EP members, followed by formal approval of the guidelines from the ClinGen SVI Working Group.

Results

We present in this article the results of the BMVCEP's refinement of ACMG/AMP variant interpretation practices to apply to somatic GOF variants in mTOR pathway genes related to brain malformations. The resulting specifications for the interpretation of somatic variants in *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2* were approved by the SVI Working Group (May 14, 2021) and now serve as a framework to guide the assessment of variant pathogenicity in these genes.

A summary of all finalized criteria compared with the original ACMG/AMP criteria is presented in Supplemental Table 3. Of the 28 original ACMG/AMP criteria, 12 were determined to be not applicable for these genes (PVS1, PM3, PM4, PM6, PP1, PP3, PP4, PP5, BS4, BP1, BP3, BP6) and were excluded from the framework. Another 12 of the 28 original criteria required gene and/or disease-specific alterations (PS2, PS3, PS4, PM1, PM2, PP2, BA1, BS1, BS2, BS3, BP4, BP5). In total, 4 criteria were approved without additional modifications (PS1, PM5, BP2, BP5). One novel criterion (BP7) was added.

Exclusion of ACMG/AMP criteria

We began our specification of curation rules for variants in these genes by determining which criteria were not pertinent to these 4 genes. Because these disorders are caused by heterozygous P variants, the PM3 criterion (relevant to diseases with recessive disorders, a variant detected in trans with a known P variant) was deemed irrelevant. Segregation patterns are not applicable when interpreting these variants because we are specifically assessing cases with de novo and postzygotic variants. For the same reason, PP1 (cosegregation with disease in multiple affected family members) and BS4 (lack of segregation in affected members of a family) were deemed irrelevant.

The disease mechanism associated with P variants in these 4 genes is GOF.[3,6] Therefore, criteria specific to LOF disease mechanisms were deemed not relevant: PSV1 (null variant in a gene in which LOF is a known disease mechanism) and BP1 (missense variant in a gene in which only LOF causes disease). It is important to note that although LOF does not cause an overgrowth phenotype consistent with OCMMPG, LOF variants in these genes may result in different phenotypes. For example, *AKT3* haploinsufficiency has been associated with a postnatal microcephaly disorder.[15] Thus, benign (B) points were not assigned to truncating variants.

GOF effects are often due to residue-specific alterations, and functional characterization is crucial to determine whether a given variant results in a GOF effect. PM4 (in-frame deletions/insertions in a nonrepeat region or stop-loss variants) does not reflect this specificity well. There is a single reported indel *NM_006218.4:*c.325_327del (p.Glu110del) in *PIK3CA*, associated with a brain malformation.[16,17] Given the rarity of P indels in these 4 genes of interest and the fact that functional evidence would be required to show that an indel resulted in a GOF consequence, we determined PM4 to not be relevant to our curation. In addition, the performance of existing in silico tools is better at estimating LOF effects and is suboptimal for reliable prediction of GOF effects.[18] Thus, PP3 (computational evidence supporting a deleterious effect) was not used.

We determined that several additional criteria were not relevant for curating GOF variants in the OCMMPG group of disorders. For example, these genes are not known to have repetitive regions without a known function in the exonic regions (BP3). The PM6 criterion (de novo without confirmation of paternity and maternity) was incorporated into PS2 (de novo, both maternity and paternity confirmed), and PP4 (phenotype specific for disease with single genetic etiology) into PS4 (prior observation of a rare variant in multiple unrelated patients with the same phenotype). We adopted ClinGen's recommendation to not use clinical laboratory classification (PP5, BP6).[19]

Modification of the ACMG/AMP criteria on the basis of specific classes of criteria

Modification for postzygotic change (PS2, PS4)

Under the PS2 criteria of the original ACMG/AMP guidelines, the presence of a de novo germline variant in a gene can be considered as strong support for pathogenicity (because of the rarity of these novel events). Similar logic can apply to pathogenic somatic variants in *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2* because these are similarly rare.[6,20,21] With this in mind, we divided PS2 into 2 separate independent criteria. PS2_moderate_1 can be used if a variant is present at a detectable variant allele fraction (VAF) in a proband with the disease but is absent from parental samples with confirmed maternity and paternity. PS2_moderate_2 can be awarded if a variant is present at a detectable VAF in an affected (or lesional) tissue sample but is absent from or detected at a significantly lower VAF in another tissue (eg, if the variant is present in 5% of the brain tissue but absent from the peripheral blood or skin, then this point can be awarded). PS2 can be used at a strong level only if both the moderate criteria are met.

We added this PS2_moderate_2 criterion because showing that a variant is absent or at a lower VAF in an

Table 1 PS4 scoring on the basis of OCMMPG phenotypes

Feature	Score
Neuropathology confirmatory of a malformation of cortical development (eg, focal cortical dysplasia, polymicrogyria)	1
Neuroimaging appearance consistent with a malformation of cortical development (without neuropathology)	0.75
Neuroimaging showing at least 1 large cerebral hemisphere with cortical malformation(s)	1
Macrocephaly (≥2 SD) and developmental delay or intellectual disability with cortical malformation	1
Macrocephaly (≥2 SD) and developmental delay or intellectual disability without cortical malformations	0.75
Clinical diagnosis of megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome or megalencephaly-capillary malformation-polymicrogyria syndrome	1
Cells from patient-derived tissue show an aberrant cell growth phenotype or increased phosphorylation (can only be used once per variant)	1
Segmental overgrowth or vascular malformation of a limb or region of the body	0.75
Presence of this variant in a tumor sample (databases such as COSMIC can be used)	0.25

COSMIC, Catalogue Of Somatic Mutations In Cancer; *OCMMPG*, overgrowth syndrome and/or cerebral malformations due to abnormalities in mTOR pathway genes.

unaffected tissue has long been accepted evidence in the research arena that a variant arose postzygotically.[22,23] Prior studies show that P somatic variants in these 4 genes are consistently detected at higher VAFs in lesional tissue (brain tissue from an FCD/hemimegalencephaly) than in nonlesional tissue (blood/ saliva from the same patient).[7,8,24] These genes are important during the development of multiple cell types, and P variants in these genes would be expected to result in a phenotype when present in non–brain tissues.[16,22] The 4 genes described in this article are expressed ubiquitously and are thought to have biological effects in most tissues. We acknowledge that, in general, somatic P variants may not cause a phenotype in all tissues, for instance, if gene expression is restricted to only a subset of tissues.

The PS4 criterion captures how many unique, unrelated probands are reposted in association with a given variant. This criterion is particularly important in our case because many variants in these 4 genes are recurrent. Because P variants within the OCMMPG genes can cause a wide phenotypic spectrum, which can vary depending on when during development the variant arose and in what tissues,[7,25] we created a table (Table 1) of comprehensive phenotypes associated with P variants in these genes.

To apply PS4 within our framework, a variant must first not be present in more than 1 individual (≤1) in control databases. Then each variant can be assigned to a category matching the phenotype of the individual in whom the variant was detected (if there are multiple possibilities, choose the single category that is associated with the highest point value). The total score obtained after consideration of all reported cases will determine the strength assigned to PS4 (see scale in Table 2, ranging from PS4 very strong to PS4 supporting).

Two additional modifications to the standard application of PS4 are worth pointing out. The first is the inclusion of the presence of the variant in cancer/tumor tissues as a potential phenotype. These 4 genes are oncogenes, and the detection of a variant in cancer/tumor samples has been used as evidence for pathogenicity.[3] So as not to weigh this type of evidence too heavily, each detection of a variant in primary cancer/tumor sample in tumor databases such as Catalogue Of Somatic Mutations In Cancer, is assigned a quarter of a point. Furthermore, we stipulated that (1) tumor data in isolation (ie, without the presence of a proband with an OCMMPG phenotype) cannot ever provide more than supporting evidence and (2) a maximum of 9 tumor samples can be counted for any individual variant. These limits protect the PS4 criterion from becoming driven entirely by tumor data alone, given the availability of thousands of tumor samples in databases.

Second, we also modified PS4 to incorporate results of functional assays that use patient-derived cells/tissue to associate a given variant with a specific cellular or biochemical phenotype; for instance, showing an aberrant cell overgrowth phenotype or increased phosphorylation of mTOR pathway targets (eg, phosphor-S6 protein). This criterion can only be applied once for a given variant.

Population-based specifications (BS1, BA1, PM2)

The population-based specifications BS1, BA1, and PM2 are designed to set thresholds for the interpretation of variants based on comparing their allele frequency against the rarity of the disorder in question. Disorders in the OCMMPG category are considered rare, but their exact prevalence is unknown. Using estimates of the clinical prevalence of FCD as a guide, the BMVCEP conservatively estimated the maximum allele frequency for any given causative P OCMMPG variant to be .0185%. FCD was chosen because it is the most common of the brain malformations associated with the 4 genes we have included in this report. On the basis of this estimate, we consider an allele frequency of >0.019% as strong evidence supporting

Table 2 PS4 strength modification scale

Supporting (PS4_P)	Moderate (PS4_M)	Strong (PS4_S)	Very strong (PS4_VS)
.5-1.25 points	1.5-3.25 points	3.5-15.75 points	≥16 points

M, moderate; *P*, suPporting; *S*, strong; *VS*, very strong.

that a variant is B (BS1, allele frequency is greater than expected for the disorder). The calculation and rationale for the estimation are provided in Supplemental Table 4. An allele frequency of >0.093% (lowered from 5%, which is consistent with the previous 5× threshold set forth in the original ACMG/AMP guidelines) is considered very strong evidence a variant is B (BA1).

PM2, a criterion to support variant pathogenicity based on its absence, was downgraded in our framework to a supporting strength point, in compliance with the SVI Working Group recommendations (https://www.clinicalgenome.org/site/assets/files/5182/pm2_-_svi_recommendation_-_approved_sept2020.pdf). We also modified PM2 to stipulate that it could still apply if a variant was present in 1 individual (≤1) in gnomAD. This modification was implemented to account for the possibility of false calls due to sequencing/calling errors,[26] the presence of cancer cohorts in the data sets, the fact that these 4 genes are strongly oncogenic and are frequently found mutated in tumor samples,[9,27] and theoretically could also be found to be enriched in blood because of clonal hematopoiesis.[28] Finally, allowing for 1 individual in control databases is consistent with what has been considered appropriate for other neurodevelopmental disorders.[29]

Proband information (PS1, PM5, BS2, BP2 BP5)

PS1 (same amino acid change as a previously-established P variant), PM5 (novel missense change at an amino acid in which a different missense change was determined to be P), BP5 (variant found in a case with an alternative molecular basis for the disease) remain unchanged, but additional specifications were provided for BS2 (observed in a healthy adult individual for disorder, with full penetrance expected at an early age), and BP2 (observed in trans with a P variant for a fully penetrant dominant gene/disorder or observed in cis with a P variant in any inheritance pattern). For BS2, clinical laboratories are encouraged to accumulate 3 or more well-phenotyped family members before applying this strong criterion. To be considered for this point, the variant should be either germline (most common) or somatic in a relevant tissue. We also considered (≥3) homozygous occurrences in gnomAD or Exome Aggregation Consortium to count for this point because P variants in these genes are heterozygous. For BP2, these disorders are caused by heterozygous alleles, therefore, this criterion can be awarded to any variant observed in cis or trans with a known P variant in the same gene.

Functional specifications (PS3, BS3, PM1, BP4, BP7)

For PS3 (well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product) and BS3 (well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing), we followed the system developed by Brnich et al[30] to add specifications for minimum quality metrics required for in vitro functional assays. The EP first conducted literature reviews and identified the most prevalent categories of functional assays used for these genes: phosphorylation of mTOR pathway targets (8 publications), DEPTOR binding (3 publications), cell survivability (2 publications), and cell proliferation (2 publications) (Supplemental References 5-13). For each assay, a review of the literature was used to define quality criteria by which the strength of the evidence provided could be assessed, including the number of basic controls, technical replicates, positive controls (variants classified as P/likely P [LP], independent of the PS3 criterion), and negative controls (variants classified as B/likely B [LB], independent of the BS3 criterion). Two separate sets of requirements were created: one for the in vitro cell line assays and another for growth-based assays using tumor samples (Supplemental Tables 5-8). Following Brnich et al[30] and the SVI Working Group's recommendation, we specified that a given assay needs to have 8 to 34 validation control variants for moderate evidence under PS3 or BS3, and 35+ control variants for strong evidence. Additional rules were established for assays involving animal models for which the recommendations by Brnich et al[30] does not apply (animal models generated with the variant of interest expressed in neural progenitors showing a complementary brain phenotype can be used at PS3 strong and animal models generated with the variant of interest expressed in non–neural tissues showing an increased cancer burden can be used at PS3 moderate).

Literature review also informed our implementation of PM1 (located in a mutational hot spot and/or critical and well-established functional domain), with empirical evidence supporting the functional domains listed in Table 3.[5,8,10,31-34] Given the relatively large size of these functional domains, however, the strength of this criterion was lowered from moderate to a supporting level (note that hot spot recurrent variants are covered by PS4).

The application of BP4 (multiple lines of computational evidence suggest no effect on gene or gene product) is hindered by the fact that existing algorithms are generally tuned for predicting LOF consequences.[18] Consequently, our use of in silico functional models for BP4 was limited to the interpretation of variants that do not affect the coding protein sequence, such as synonymous, intronic positions (except canonical splice sites), and noncoding variants in the untranslated regions. For these variants, if 2 out of 3 splicing prediction tools (varSEAK, spliceAI, and MaxEntScan) predicted no effect on splicing function, then BP4 could be applied.

Nucleotide conservation was also taken into consideration for silent variants. For synonymous, intronic positions (except canonical splice sites), and noncoding variants in the untranslated regions, if the nucleotide is not conserved, BP7 can be applied. Not conserved variation is defined as the same nucleotide not present in all vertebrates or with a PhyloP score < 0.1.

Table 3 Critical functional domains for the canonical transcripts in *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2*

Gene	*AKT3*	*MTOR*	*PIK3CA*	*PIK3R2*
Transcript	NM_005465.4	NM_004958.3	NM_006218.3	NM_005027.3
Domain	Pleckstrin homology domain AA: 5-109 g.244006460-243809297	Kinase domain AA: 1382-1982 g.11259424-11188148	Kinase Ras-binding domain AA: 173-292 g.178917642-178921394	Sequence homology 2 domain AA: 328-716 g.18273092-18280065
Domain	Catalytic kinase domain AA: 151-388 g.243801023-243708899	FKBP-rapamycin-binding domain AA: 2015-2114 g.11187854-11187076	Kinase domains AA: 322-483 and 797-1068 g.178921482-178928263 g.178942582-178952149	
Domain	C-terminal protein kinase AA: 425-475 g.243675707-243668566		Adaptor binding domain AA: 31-108 g.178916704-178916937	

Amino acid and genomic coordinate boundaries of critical functional protein domains employed in this curation are listed.
AA, amino acid; *g.*, genomic coordinate.

Performance of the BMVCEP ACMG/AMP specifications in variant classification

We validated our modified ACMG/AMP criteria using 43 variants from our 4 genes. Application of our framework led to 4 of these variants being classified as B, 12 LB, 3 LP, 14 P, and 10 variants of uncertain significance (VUS). The 17 P/LP variants encompass most of the somatic variants reported in the literature for these genes. A detailed description of the variants and points assigned to derive the criteria is provided in Supplemental Table 9. After each variant was scored using our modified criteria, an overall classification was assigned using a previously published Bayesian-based scoring system.[35]

We compared our 43 classifications with interpretations previously submitted to ClinVar. Approximately half of the variants we curated had not been previously entered into ClinVar (20/43). Of the 17 variants we curated as P/LP, 4 were absent from ClinVar, and 8 of the remaining 13 had 0 stars. Of the 4 B variants we curated, 1 was absent from ClinVar and 3 remained B. Of the 12 LB variants curated, 7 were absent from ClinVar, 4 remained LB, and 1 reclassified from B. Of the 10 VUS variants curated, 8 were absent from ClinVar, 1 was reclassified from likely benign, and 1 reclassified from benign. In addition, 2 variants reported in the literature as P, but not previously entered into ClinVar, were classified as VUS: NM_004958.4:c.4375G>T (p.Ala1459Ser) in *MTOR*[36] and c.93A>G (p.Ile31Met) in PIK3CA.[37] All finalized interpretations have been submitted to ClinVar under the BMVCEP assertion level (3 star).

Limitations

The criteria are conservative to minimize false-positive interpretations. Further refinement over time may be necessary. Unpublished data from clinical laboratories were not used, but we recognize that this is an important source of information.

The available functional literature failed to provide evidence above a supporting level largely because of the lack of validated control samples tested. Our variants were highly recurrent and therefore, case-level data were sufficient to determine pathogenicity. However, novel variants are being discovered in mTOR pathway genes and will need to use functional evidence for pathogenicity. This highlights the need to develop additional high throughput functional assays to systematically interrogate the effects of all possible missense variants in the mTOR pathway genes.

Finally, we recognize that, in practice, there may be substantial heterogeneity in how Clinical Laboratory Improvement Amendments approved laboratories identify high confidence somatic variants. There is a need to establish consensus biological, experimental, and statistical methods and parameters (eg, sequencing platform, confirmation of somatic variants using sequencing on orthogonal platforms, minimum read depth, VAF, tissue source, preservation method).

Discussion

The development of approaches for efficient, rigorous, and reproducible variant interpretation is critical to the field of genomic medicine. The 2015 release of the ACMG/AMP criteria provided a critical foundation for diagnosing individuals with genetic conditions associated with LOF germline variants. This study expanded this foundation by extending it to GOF somatic genetic variants, beginning with those arising in a collection of genes associated with mTORopathies: *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2*. In doing so, the BMVCEP amendments to the ACMG/AMP criteria presented herein provide a useful initial framework for standardizing the interpretation of other conditions associated with somatic genetic variation, providing a more unifying approach for these types of postzygotic disorders. Several innovative principles developed in this study could

be applied to other somatic or GOF disorders, including using VAF differences as a proxy for de novo variation, incorporating both somatic and germline evidence, and delineating phenotype differences dependent on variable tissue expression.

Somatic variants at increasingly lower allelic fractions are being identified and reported as causal for an increasing number of disorders. Variants in mTOR pathway genes provide a unique perspective because even very small allelic fractions result in recognizable lesions in the brain. However, for other disorders such as neurodegeneration and autism spectrum disorders, it is more difficult to conclusively say that variants present at small allelic fractions can result in more global phenotypes.[38,39] Using the framework delineated earlier with disease-specific modifications would aid in the pathogenicity classification of variants in these other disorders. Furthermore, the method of a collaborative systematic review of available literature to develop rigorous standards for the evaluation of experimental results will be very important for such groups.

Experimental functional assays can be especially useful in the interpretation of somatic GOF variants. These BMVCEP guidelines specify minimum requirements needed for data from functional assays to be used to support variant classification, including deployment of appropriate positive and negative experimental controls. Adoption of these recommendations by research laboratories will promote translatability between experimental findings, genomic science, and clinical care. Somatic variation implicated in other disorders can also use the minimum requirements and validation controls to develop novel functional assays to provide further evidence for pathogenicity.

Finally, we note that there is an urgent need to translate genetic discoveries in patients for treatments. Because mTORopathies are potentially treatable with mTOR inhibitors, implementation of this framework will help ensure that patients with pathogenic variants in the *AKT3*, *MTOR*, *PIK3CA*, and *PIK3R2* can be efficiently and accurately identified for emerging clinical trials.

Data Availability

The Brain Malformation Variant Curation Expert Panel submitted all variants to ClinVar with evidence summaries detailing what data was used for each interpretation. A comprehensive list of variants and their ClinVar identifiers can be found in Supplemental Table 9.

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Author Information

Conceptualization: A.P., T.Y.; Data Curation: A.L., M.E., E.Y., C.J.Y., L.S., C.M.E.A., G.Mo., L.H.R., S.S., M.P., M.D.; Formal Analysis: A.L., M.E., E.Y., C.J.Y., L.S., C.M.E.A., G.Mo., L.H.R., S.S., M.P., M.D., A.P., T.Y.; Funding Acquisition: A.P., T.Y.; Investigation: A.L., A.S., M.E., E.Y., C.J.Y., L.S., C.M.E.A., G.Mo., L.H.R.; Project Administration: A.L., D.K. Software: A.S.; Supervision: Y.C., Y.-S.L, A.J.B., R.G., H.C.M., C.A.W., G.Mi., D.T.M., T.Y., A.P.; Writing-original draft: A.L., T.Y., A.P.; Writing-review and editing: A.L., A.S., C.M.E.A., A.J.B., Y.C., M.D., M.E., R.G., D.K., Y.-S.L., H.C.M., D.T.M., G.Mi., G.Mo., L.H.R., M.P., L.S., S.S., C.A.W., E.Y., C.J.Y., T.Y., A.P.

Ethics Declaration

Our study curates already de-identified gene variants collected from publicly available sources only, namely ClinVar and primary literature in PubMed, and does not include individual-level data or any protected health information. As such, it does not constitute human subject research. Appropriate consent for the initial publication of the data used is expected to have been obtained by the primary authors and/or submitters to ClinVar.

Conflict of Interest

D.T.M. has received honoraria from Ambry Genetics and PreventionGenetics. All other authors declare no conflicts of interest.

Additional Information

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Affiliations

[1]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA; [2]Epilepsy Genetics Program, Division of Epilepsy and Clinical Neurophysiology, Department of Neurology, Boston Children's Hospital, Boston, MA; [3]Department of Pediatrics, Harvard Medical School, Boston, MA; [4]Department of Neurology, Harvard Medical School, Boston, MA; [5]Department of Radiology, University of California, San Francisco, CA; [6]Department of Pathology and Immunology, Washington University School of Medicine in St. Louis, St. Louis, MO; [7]Broad Institute of MIT and Harvard, Cambridge, MA; [8]Precision Health Program, Geisinger, Danville, PA; [9]Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Department of Neuroscience, Meyer Children's University Hospital, University of Florence, Florence, Italy; [10]Center for Pediatric Neurological Disease Research, St. Jude Hospital, Memphis, TN; [11]Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA; [12]Department of Pediatrics, University of Washington, Seattle, WA; [13]Division of Reproductive Genetics, Northwestern Medicine, Chicago, IL; [14]Department of Radiology, Boston Children's Hospital and Harvard Medical School, Boston, MA

Members of ClinGen Brain Malformation Variant Curation Expert Panel

Christelle Achkar, James Barkovich, Yang Cao, Jamel Chelly, Marina DiStefano, Elizabeth Engle, Michael Evenson, Renzo Guerrini, William Hong, Hyunyong Koh, Devon Knight, Abbe Lai, Rhonda Lassiter, Yi-Shan Lee, Eric Marsh, Heather C. Mefford, David T. Miller, Ghayda Mirzaa, Ganesh Mochida, Rebecca Pinsky, Annapurna Poduri, Lance H. Rodan, Mayher Patel, Catherine Shain, Lacey Smith, Aubrie Soucy, Sara Spencer, Christopher A. Walsh, Edward Yang, Timothy Yu, Bo Yuan, Christopher Yuskaitis.

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