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ARTICLE

Expanding the spectrum of *TUBA1A*-related cortical dysgenesis to Polymicrogyria

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De novo **mutations in the *TUBA1A* gene are responsible for a wide spectrum of neuronal migration disorders, ranging from lissencephaly to perisylvian pachygyria. Recently, one family with polymicrogyria (PMG) and mutation in *TUBA1A* was reported. Hence, the purpose of our study was to determine the frequency of *TUBA1A* mutations in patients with PMG and better define clinical and imaging characteristics for *TUBA1A*-related PMG. We collected 95 sporadic patients with non-syndromic bilateral PMG, including 54 with perisylvian PMG and 30 PMG with additional brain abnormalities. Mutation analysis of the *TUBA1A* gene was performed by sequencing of PCR fragments corresponding to *TUBA1A*-coding sequences. Three *de novo* missense *TUBA1A* mutations were identified in three unrelated patients with PMG representing 3.1% of PMG and 10% of PMGs with complex cerebral malformations. These patients had bilateral perisylvian asymmetrical PMG with dysmorphic basal ganglia cerebellar vermian dysplasia and pontine hypoplasia. These mutations (p.Tyr161His; p.Val235Leu; p.Arg390Cys) appear distributed throughout the primary structure of the alpha-tubulin polypeptide, but their localization within the tertiary structure suggests that PMG-related mutations are likely to impact microtubule dynamics, stability and/or local interactions with partner proteins. These findings broaden the phenotypic spectrum associated with *TUBA1A* mutations to PMG and further emphasize that additional brain abnormalities, that is, dysmorphic basal ganglia, hypoplastic pons and cerebellar dysplasia are key features for the diagnosis of *TUBA1A*-related PMG.**
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INTRODUCTION

TUBA1A-related cortical dysgeneses (MIM *602529) are known to be represented by a spectrum of agyria/pachygyria malformations associated with a hooked aspect of the frontal horn of the lateral ventricles and dysgenesis of the anterior limb of the internal capsule, a thin corpus callosum and hypoplasia of pons and cerebellum.[1–4] In addition to agyria/pachygyria, one patient with the *TUBA1A* mutation p.Arg390Cys was described with a milder presentation designated as a 'simplified' gyral pattern.[3] More recently, Jansen *et al*[5] reported one family with polymicrogyria (PMG) in whom a novel *TUBA1A* mutation was identified. In this family, the mutation was transmitted by the asymptomatic mother who carried somatic mosaicism for the c.13A>C (p.Ile5Leu) missense mutation. Both sisters showed PMG, bilateral perisylvian in one case and focal in the second, with dysmorphic basal ganglia and variable degrees of brainstem and cerebellar hypoplasia.

PMG, including the most common perisylvian subtype, represents a heterogeneous group of disorders characterized by an excessive number of abnormally small gyri that produces an irregular cortical surface and a disorganized cortical lamination. The incidence of PMG is unknown, largely because of the clinical and etiological heterogeneity, and the misdiagnosis related to the paradoxical smooth appearance of the pial surface that is often confounded with pachygyria. Recent advances in imaging investigations have improved the diagnosis and classification of these conditions and several subtypes of PMG are recognized based on differences in extent and topography.[6] Genetic aetiologies of PMG are various and include chromosomal deletions and duplications[7] and mutations in several genes, such as *SRPX2*,[8] *GPR56*,[9] *TUBB2B*,[10] *TUBB3*,[11] *PAX6*,[12] *TBR2*,[13] *KIAA1279*,[14] *NHEJ1*,[15] *RAB3GAP1*,[16] *TUBA8*[17] and *TUBA1A*[5] with all but *GPR56, TUBA1A, TUBB3* and *TUBB2B* found in rare syndromes. However, it remains unclear how frequently *TUBA1A* mutations are found in patients with PMG.

Tubulin alpha1A (TUBA1A) is a critical structural subunit of microtubules that is transiently expressed during neuronal development.[18] The TUBA1A protein is formed by a core of two β-sheets surrounded by α-helices and can be divided into three functional domains: an N-terminal domain (residues 1–205) that contains the

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guanine nucleotide-binding region, an intermediate domain (residues 206–381) involved in interprotofilament lateral contacts and the regulation of tubulin curvature, and a C-terminal domain (residues 382–451) that constitutes the binding surface for defined microtubule-associated proteins (MAPs) and molecular motors, such as kinesins and dynein.[19–21]

Here, we report a *TUBA1A* gene analysis in a large cohort of PMG patients to investigate how common *TUBA1A* mutations are in patients with PMG and to identify the key phenotypical features that could guide the diagnosis. We identified three *TUBA1A* mutations, of these two are novel, representing 3.1% of children with PMG and 10% of children with PMG with complex cerebral malformations. Structural data suggest that PMG-associated mutations of *TUBA1A* may operate *via* an impairment of local interactions with partner proteins by modifying a relevant protein–protein interface.

SUBJECTS AND METHODS

From our large cohort of subjects with unexplained cortical malformations (MCD) documented by a review of brain imaging (available for all subjects and reviewed by NBB and NB) and for whom DNA samples were available ($n = 750$), we selected 95 sporadic patients with non-syndromic bilateral PMG (34 females and 61 males), 54 (57%) with perisylvian PMG (14 females and 40 males), 20 (21%) had generalized PMG, 5 (5%) had frontal or fronto-parietal PMG, 4 (4.2%) had PMG and heterotopia (2 males and 2 females); 12 (12.6%) had multifocal bilateral PMG (patchy PMG in both hemispheres, without any particular pattern or gradient).

MCD were diagnosed and classified according to the recent classification criteria proposed by Barkovich *et al*.[22] PMG is diagnosed according to the three distinguishing criteria (ie irregular surface of cortex, thickened or overfolded cortex aspect, and irregularity at the gray–white interface). Pachygyria is one of the entities of the lissencephaly spectrum characterized by few broad and gyri. Both, PMG and pachygyria, are also classified according to their main topographies, that is, perisylvian PMG or perisylvian (or central) pachygyria. Simplified gyral pattern is defined by reduced number and shallow appearance of gyri and normal-to-thin cortex.

Of the selected 95 patients with PMG, 30 had additional brain abnormalities consisting of partial or complete agenesis of corpus callosum, dysmorphic basal ganglia, brainstem hypoplasia and/or cerebellar hypoplasia or dysplasia. For all patients, known causes of PMG were excluded, including metabolic disorders such as peroxisomal syndromes.

Molecular analysis

DNA was extracted from blood samples using standard protocols and CGH-array investigations were performed for all patients. CGH-array and screening for mutations in *GPR56*, *TUBB2B* and *TUBB3* were negative in all cases. Mutation analysis of the coding exons of *TUBA1A* were performed by direct sequencing of genomic DNA as described previously.[2,21]

Clinical information

All patients found to be mutated for *TUBA1A* are under regular pediatric neurology follow-up – and were known personally to at least one of the authors and were re-examined for the purpose of the study. Clinical information was either collected directly at the Pediatric Neurology unit of Necker Enfants Malades Hospital, Paris-Descartes University, or obtained from the referring physicians.

Clinical data and blood samples were obtained following informed consent of parents and/or patients.

RESULTS

TUBA1A mutations are identified in 3.1% of unexplained PMG and 10% of PMG with complex cerebral malformations

We screened the complete *TUBA1A*-coding exonic sequences and their flanking 5′ and 3′ regions using bi-directional Sanger sequencing of PCR fragments corresponding to *TUB1A1* exons in 95 patients with PMG, including 30 with complex cerebral malformations.

We identified three missense mutations in *TUBA1A* in three patients with PMG (3 out of 95; 3.1%). This frequency of *TUBA1A* mutations shifts to 10% (3 out of 30) when considering the subset of patients with PMG with additional brain abnormalities, consisting of partial or complete agenesis of corpus callosum, dysmorphic basal ganglia, brainstem hypoplasia and/or cerebellar hypoplasia or dysplasia.

For patient 1, *TUBA1A* analysis showed a c.703G>T substitution leading to a p.Val235Leu mutation in the intermediate domain of the protein. DNA analysis of patient 2 showed a c.481 T>C substitution leading to a p.Tyr161His mutation in the N-terminal domain; and patient 3 had a c.1168 C>T substitution leading to a p.Arg390Cys mutation in the C-terminal domain. In each patient, the mutation was confirmed to be *de novo* by direct sequencing of both parents' DNA. The first two mutations are novel and the third one, p.Arg390Cys (c.1168 C>T), was previously reported in Kumar *et al*[3] in a patient with simplified gyral pattern. These mutations were found neither in the parents' DNA nor in more than 300 chromosomes from normal subjects.

Upon identification of the mutations, patient 1 (p.Val235Leu (c.703G>T) was a 7.5-year-old boy, the fourth child of non-consanguineous parents. He was born full-term with normal neonatal parameters. During the first year of life, he was mildly delayed with autistic features. At 2.5 years old, he was referred to a pediatric neurological department for refractory left focal status epilepticus. At the most recent evaluation, his head circumference was normal (70th percentile), language was echolalic and right hemiparesis with hemianopsia were pointed out. His epilepsy was drug resistant with up to 7 partial seizures per day. Brain magnetic resonance imaging showed bilateral and asymmetrical perisylvian PMG more prominent in the right perisylvian region and frontal region with dysmorphic basal ganglia and moderate hypoplasia of the corpus callosum. The cerebellum and brainstem were normal (Figures 1a–d).

Patient 2, an 11-year-old girl with the mutation p.Tyr161His (c.481 T>C), was the first child of non-consanguineous parents, born at term with normal neonatal growth parameters. From birth, she was very hypotonic and had poor visual contact. Occipital seizures started at 3 months of age and became progressively refractory to anti-epileptic drugs. At the last evaluation, her head circumference was 44 cm (<3rd percentile), she was able to walk with aid, but had a left hemiparesis. She had no language and was cortically blind. Magnetic resonance imaging showed bilateral and asymmetrical perisylvian PMG more localized in right perisylvian region with dysmorphic basal ganglia, a dysplastic cerebellar vermis, hypoplastic pons and moderate hypoplasia of the corpus callosum. In addition, there was right occipital atrophy with ulegyria (Figures 1e–h).

Patient 3, a 1-year-old boy with the mutation p.Arg390Cys (c.1168 C>T), was, the first child of non-consanguineous parents, born at term with normal neonatal growth parameters. He was initially referred for poor eye gaze and pursuit at 3 months. On examination, he showed microcephaly and extreme hypotonia. On clinical reevaluation at 1.1 years, he had developed convergent strabismus with pyramidal signs and spasticity. Magnetic resonance imaging showed asymmetrical perisylvian PMG, localized in the left perisylvian region, while on the right, it extended toward the parietal region with dysmorphic basal ganglia, a dysplastic cerebellar vermis, severe hypoplasia of brainstem and corpus callosal hypoplasia (Figures 1i–l).

Structural predictions for *TUBA1A* mutants

To predict potential functional consequences of the mutations on *TUBA1A* functions, we determined the localization of each mutation,

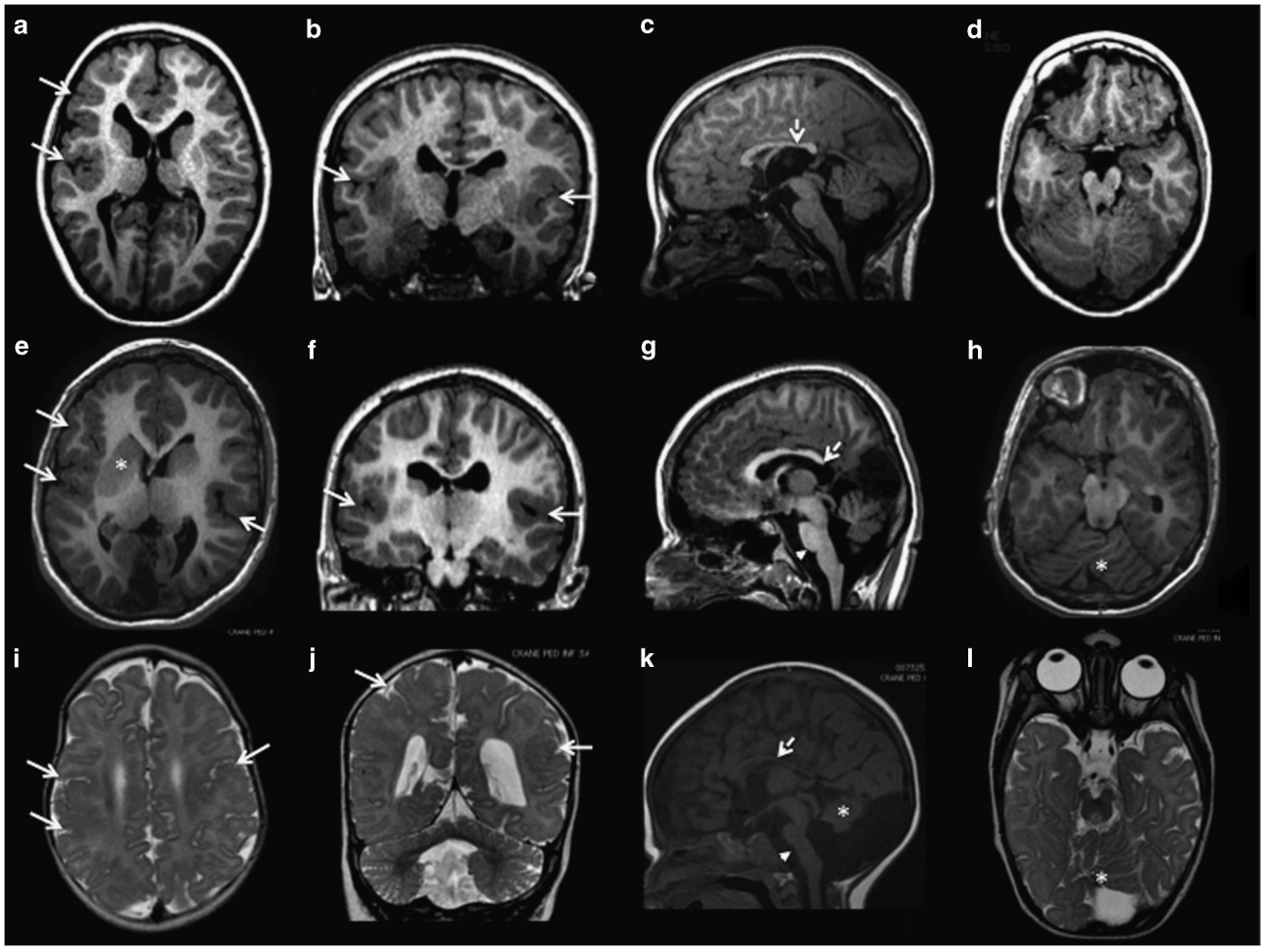

Figure 1 Brain magnetic resonance imaging (MRI) of the three patients with *TUBA1A* mutations. (**a**–**d**) Brain MRI of patient 1 at the age of 5 years; (**e**–**h**) brain MRI of patient 2 at the age of 11 years; (**i**–**l**) brain MRI of patient 3 at the age of 12 months. This figure shows different aspects of PMG in axial (**a**, **e**, **i**) and coronal (**b**, **f**, **j**) sections. All three patients show asymmetrical PMG that appears more prominent in right perisylvian and frontal regions (arrows in **a**–**f**). Associated malformations include mild (arrowhead in (**g**)) or severe brainstem hypoplasia (arrowhead in (**k**)), vermian dysplasia (asterisks in (**h**, **k** and **l**)), moderate (dotted arrows in (**c** and **g**)) or severe hypoplasia of the corpus callosum (dotted arrows in (**k**)). The basal ganglia are malformed, appearing as large round structures in which the caudate, putamen and globus pallidus cannot be distinguished (asterisks in (**e**)).

including the mutation p.Ile5Leu previously described in PMG.[5] Mutated residues (Tyr161, Val235 and Arg390) were localized in alpha-helices (respectively helix H4, in the N-terminal domain, helix H7, at the beginning of the intermediate domain, and helix H11 in the C-terminal domain) and the Ile5 residue in a beta-strand B1 (Figure 2). Arg390 is an exposed residue on the C-terminal domain of tubulin, known to form the interface with a number of MAPs and motors, in particular kinesins. Ile5 and Tyr161 are partially buried residues closely localized in the tertiary structure, they both contact helix H3, which is likely involved in lateral interactions between microtubule protofilaments,[20,23] thus their substitution (p.Ile5Leu and p.Tyr161His) might influence microtubule assembly and dynamics. p.Val235Leu affects a buried residue on helix H7, which might alter the overall folding of TUBA1A. However, because this mutation only adds one carbon to the aliphatic side chain, p.Val235Leu might have a more subtle effect on the piston movement of helix H7, known to control the transition of curved-straight tubulin and thus may alter microtubule dynamics or stability.[24] According to structural data, none of these mutations seem to lie in the nucleotide-binding pocket of alpha-tubulin suggesting that they may not influence GTP binding. Additionally, they are not located at the intradimer interface, suggesting that they would not be involved in the alpha-beta heterodimerization process. Altogether, these results show that PMG-related mutations are different from those associated with agyria/pachygyria and suggest that they might specifically impact microtubule dynamics or stability, or local interactions with partner proteins, such as MAPs and motor proteins (kinesins or dynein) by modifying a relevant protein–protein interface.

DISCUSSION

One gene, several phenotypes

Previous data underlined the importance of *TUBA1A*-related perisylvian pachygyria with dysmorphic basal ganglia, observed in at least 4/11 (36.3%) patients, many of which with the recurrent mutation p.Arg264Cys.[1,21] In this study, we provide additional evidences showing that *de novo* missense mutations in the *TUBA1A* gene are causative for perisylvian PMG, further expanding the spectrum of *TUBA1A*-related cortical dysgenesis. Two of these three mutations involved in PMG are novel, and the remaining one was previously

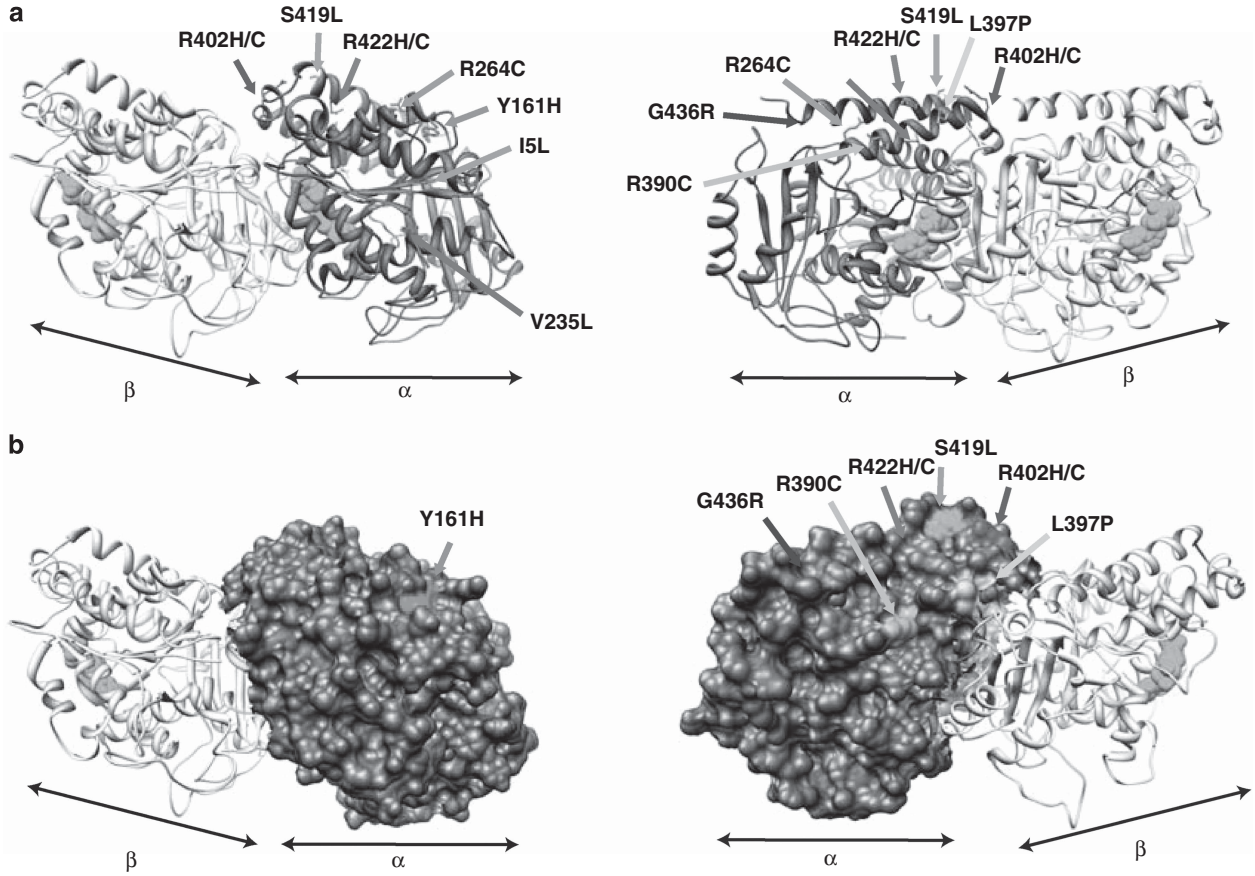

Figure 2 Localization of *TUBA1A* mutations. (**a**) Atomic structure of the alpha-beta tubulin heterodimer (PDB ID 1JFF;[19]) with both alpha- (dark gray) and beta tubulin (light gray) displayed as ribbons. Displayed in sticks and labeled are the side chains of Ile5, Tyr161, Val235 and Arg390, as well as other reported *TUBA1A*-mutated residues associated with agyria/pachygyria: Ile188, Arg264, Leu397, Arg402, Ser419, Arg422 and Gly436. The tubulin-bound GTP and GDP molecules are represented as cyan spheres. (**b**) Same as (**a**) with alpha-tubulin displayed as a molecular surface.

reported in a patient with 'simplified' gyral pattern.[3] Interestingly, the perisylvian location of predilection of PMG corresponds to the most frequent location of pachygyria in *TUBA1A*-related cortical dysgeneses.

Patients reported here with *TUBA1A*-related PMG demonstrate the hallmarks of PMG: the irregularity of the surface of the cortex, the aspect of cortex that appears thickened or overfolded, and the irregularity at the gray–white interface. Clinically, patients with *TUBA1A*-related PMG developed early onset seizures and refractory epilepsy in two of the three cases. This also distinguishes them from those with pachygyria. Additionally, they showed less clinical features of pseudobulbar paresis when compared with those with pachygyria. To some extent, *TUBA1A*-related PMG shares some common features with PMG related to *TUBB2B*, a beta tubulin found to be involved in PMG.[10,25] In both conditions, large and dysmorphic basal ganglia, a hypoplastic brainstem and cerebellar hypoplasia are the hallmarks of tubulin-related cortical dysgenesis. However, *TUBB2B*-related PMG differs from *TUBA1A*-related PMG by its more diffuse presentation and its topography most prominent in anterior regions, that is, fronto-parieto temporal PMG.[10]

While *TUBA1A* mutations were found in 3.1% of patients with perisylvian PMG, our results suggest that *TUBA1A* is a gene responsible for complex cortical malformations that include PMG rather than a gene for isolated perisylvian PMG. In all three cases, PMG was limited to the perisylvian region and was combined with the most significant features commonly reported in *TUBA1A*-related cortical dysgenesis, that is, dysmorphic basal ganglia lacking clear separation between the caudate and putamen, as well as cerebellar and corpus callosal abnormalities.

TUBA1A mutations lead to pachygyria and PMG

As far as *TUBA1A* mutations are concerned, our results further argue for a continuum between classic lissencephaly, perisylvian pachygyria and perisylvian PMG. Indeed, whether it is PMG or agyria/pachygyria, most of the patients with mutations in *TUBA1A* have also corpus callosum, anterior arm of internal capsule, basal ganglia, cerebellum and brainstem development abnormalities. The presence of these common abnormalities suggests that TUBA1A-related PMG/pachygyria/lissencephaly are malformations of a same spectrum. According to current classifications, lissencephaly – that is, the agyria–pachygyria spectrum – results from abnormalities of neuronal migration, while PMG is suspected to result from abnormalities during late neuronal migration or early cortical organization.[22,26] However, these major steps that govern cortical organization are not temporally separate; proliferation continues after migration begins, and migration continues as organization commences. Moreover, recent evidence from the studies of tubulin (*TUBB2B*) suggest that the pathogenesis of PMG is more complex and could also be related to alteration of regulation of pial basement membrane integrity during development, a mechanism reminiscent of

cobblestone lissencephaly.[27] We previously showed by biochemical and cellular studies that disease-associated mutations in *TUBA1A* result in a spectrum of defects in the tubulin folding and heterodimer assembly pathway and microtubule dynamics.[28] Some other mutations are predicted to interfere with MT's partners, such as MAPs, including DCX, and motor proteins such as kinesin. This suggests either a loss-of-function or dominant-interfering mechanism to explain how mutations in this ubiquitous cytoskeletal protein could give rise to a specific neuronal migration defects. Here, mutated residues associated with PMG, (ie, the two mutations reported here and the two previously reported) are different from those associated with agyria/pachygyria spectrum and structurally predicted to be involved in lateral interactions between protofilaments, microtubule dynamics or interactions with tubulin partners. Although PMG mutations follow the trend of the majority of other mutations in *TUBA1A* gene, which predominantly affect surface residues,[29,30] one can hypothesize that phenotypic differences may result from mutation-specific alterations of binding sites with other partners or the function of microtubules themselves. It is worth noticing that the mutation p.Arg390Cys located on a surface residue of the C-terminal region of TUBA1A, and suspected to result in impairments of interactions with partner proteins, leads to two distinct phenotypes: PMG in our series and 'simplified' gyral pattern described in a previous report.[3] Both conditions are associated with cerebellar hypoplasia, and corpus callosum abnormalities. These two distinct cortical malformations related to the same mutation suggest that the particular genetic background might contribute to explain the phenotypic heterogeneity.

Our observations confirm that *TUBA1A* mutations can be responsible in a significant proportion of perisylvian PMGs.[5] When compared with the reported cases, our patients demonstrate the most significant features commonly reported in *TUBA1A*-related cortical dysgenesis, that is, dysmorphic basal ganglia lacking a clear separation between the caudate and putamen, cerebellar and corpus callosum abnormalities. Interestingly, the fact that *TUBA1A* mutations resulting in agyria/pachygyria are in most cases different from those involved in PMG provides an opportunity to study how such specific residues differentially regulate microtubule function and protein interactions.[30]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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