Brain malformations and mutations in α- and β-tubulin genes: a review of the literature and description of two new cases

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ABBREVIATIONS

MCDs	Malformations of cortical development
PMG	Polymicrogyria

AIM The aim of this study was to determine the frequency of mutations in tubulin genes (*TUBB2B, TUBA1A, and TUBB3*) in patients with malformations of cortical development (MCDs) of unknown origin.

METHOD In total, 79 out of 156 patients (41 males, 38 females; age range 8mo–55y (mean age 13y 3mo, SD 11y 2mo) with a neuroradiological diagnosis of MCDs were enrolled in the study. The 77 excluded patients were excluded for the following reasons: suspected or proven diagnosis of pre- or perinatal ischaemic insult (*n*=13); syndromic disease (*n*=10); congenital infection (*n*=14); pregnancy complicated by twin-to-twin transfusion syndrome (*n*=2); proven mutations in known genes (*n*=13); poor magnetic resonance imaging (MRI) quality, or lack of informed consent (*n*=25). A genetic analysis of the *TUBA1A*, *TUBB2B* and *TUBB3* genes was carried out by direct sequencing of the coding regions of the relevant genes for each participant. Previously described patients with mutations in the *TUBB2B* and *TUBA1A* genes were reviewed; clinical and neuroradiological findings were compared and discussed.

RESULTS Two novel heterozygous mutations were detected: a heterozygous mutation in exon 4 of the *TUBA1A* gene (c.1160C>T) in a 5-year-old female with microcephaly, severe intellectual disability, and absence of language, and a c.1080 _1084del CCTGAinsACATCTTC in exon 4 of the *TUBB2B* gene in a 31-year-old female with microcephaly, spastic tetraparesis, severe intellectual disability, and scoliosis. Different types of cortical abnormalities, cerebellar vermis hypoplasia, and optic nerve hypoplasia/atrophy were detected on MRI. Dysmorphisms of the basal ganglia and the hippocampi with abnormalities of the midline commissural structures were present in both cases.

INTERPRETATION The consistent presence of hypoplastic and disorganized white matter tracts suggests that, in addition to defects in neuronal migration, disruption of axon growth and guidance is a peculiar feature of tubulin-related disorders.

Malformations of cortical development (MCDs) are a group of brain disorders with a wide spectrum of clinical presentations ranging from hemiparesis to tetraparesis, developmental delay or intellectual disability, and, frequently, drug-resistant epilepsy.[1] MCDs may be a result of environmental causes such as congenital infections (particularly the toxoplasmosis, other infections, rubella, cytomegalovirus, herpes simplex virus [TORCH] complex),[2] localized or diffuse in utero ischaemia,[3] or genetic causes such as chromosomal rearrangements and single-gene disorders.[4] These latter are considered relevant causes of MCDs. The latest classification of MCDs is based on both magnetic resonance imaging (MRI) findings and mutations in genes involved in cortical development.[1]

Tubulin-encoding genes (*TUBA1A*, *TUBB2B*, *TUBB3*, and *TUBA8*) are highly expressed during cortical development with a specific spatial and temporal expression pattern.[5,6] They encode dimeric proteins consisting of two closely related subunits, α and β, representing the major constituents of microtubules.[7] These proteins play a key role in several cellular processes crucial for cortical development during neuronal migration, in cortical laminar organization, and in neuronal guidance of the radial glia (axon outgrowth and maintenance).[8,9] Mutations in the tubulin genes mostly affect the formation of tubulin heterodimers and result in different disorders. The associated phenotypic spectrum encompasses a wide range of brain malformations, all reflecting axon guidance disturbances

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(abnormal fascicles and axon tracts, abnormalities of the internal capsule and corpus callosum, hypoplasia of the brainstem and corticospinal tracts), and migration or post-migration defects (abnormal cortex and hippocampal lamination).[5,10]

Mutations in the *TUBA1A* gene have been associated with various grades of lissencephaly, ranging from the complete loss of gyri and sulci (agyria) to simplified abnormally thick convolutions (pachygyria), and perisylvian polymicrogyria (PMG).[11–19] Mutations in the *TUBB2B* gene have been associated with bilateral, asymmetrical PMG[20] and schizencephaly[21] as well as symmetrical PMG and pachygyria.[22,23] Axon guidance disorders (corpus callosum abnormalities and hypoplasia of the oculomotor nerves) and cortical malformations (PMG and gyral disorganization) have been found in individuals carrying defects in the *TUBB3* gene.[24,25] Mutations in the *TUBA8* gene have been associated with optic atrophy and bilateral PMG.[26]

The aims of this study were to determine the frequency of mutations in the *TUBA1A*, *TUBB2B*, and *TUBB3* tubulin genes; to describe the associated neuroradiological patterns in a sample of patients affected by MCDs of unknown origin; and to review the literature describing the spectrum of MCDs associated with mutations in the tubulin genes.

METHOD

Inclusion and exclusion criteria and classification system

In total, 79 patients (41 males, 38 females; mean age 13y 3mo, SD 11y 2mo; age range 8mo–55y) out of 156 patients with a neuroradiological diagnosis of MCDs were enrolled in the study. The 77 excluded patients were left out of the study for the following reasons: suspected or proven diagnosis of pre- and perinatal ischaemic insult (n=13); syndromic disease (n=10); congenital infection (n=14); pregnancy complicated by twin-to-twin transfusion syndrome (n=2); proven mutations in known genes (n=13); poor magnetic resonance imaging (MRI) quality or lack of informed consent (n=25).

The MRI findings of the 79 selected patients were reviewed by two neuroradiologists (FA and FT) blind to the patients' identities and clinical data and consensus was reached. On the basis of the imaging pattern, the participants were classified according to the latest MCD classification system proposed by Barkovich et al.[1] (see Table I).

Genetic analysis and neuroradiological study

A genetic analysis of the *TUBA1A*, *TUBB2B* and *TUBB3* genes was carried out by direct sequencing of the coding regions of the relevant genes for each participant. Missense changes were analysed using pathogenicity prediction programs such as SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html); PolyPhen 2 (http//genetics.bwh.harvard.edu/pph); MutPred (http://mutpred.mutdb.org/); PhD-SNP (http://snps.uib.es/phd-snp/phd-snp.html); and Pmut (http://mmb2.pcb.ub.es:8080/PMut/) (see Data S1, online supporting information).

The clinical and genetic evaluations were approved by the local ethics committee of the Eugenio Medea Scientific Institute. All participants or their caregivers gave written informed consent for the study.

What this paper adds

- We describe two new mutations in the tubulin superfamily, extending the spectrum of associated brain malformations.
- Published cases of mutations in the *TUBB2B* and *TUBA1A* genes are reviewed.

RESULTS

Genetic results

Three heterozygous mutations were detected: two mutations in exon 4 of the *TUBB2B* gene (c.419G>C [p. Gly140Ala] and c.1080 _1084del CCTGAinsACATCTTC [p.Leu361_Lys362delinsHisLeuGln]) and one mutation in exon 4 of the *TUBA1A* gene (c.1160C>T [p.Ala387Val]). No mutation was found in the *TUBB3* gene. The patient carrying one of the mutations in *TUBB2B*, c.419G>C, had already been reported in the literature.[21] Missense variants in either the *TUBB2B* or *TUBA1A* gene affect residues that are highly conserved from humans to yeast. The novel missense variant was predicted to have damaging effects by the different software used (PoliPhen2, Pmut, and PhD-SNP). A segregation analysis of the variants showed that all of them were de novo variants. We also excluded the presence of these mutations in a comparison group of 600, and in the SNP (dbSNP) and 1000 genome databases (see Fig. S1, online supporting information).

Clinical cases and review of literature

The clinical and neuroradiological data of the patients with mutations in the *TUBB2B* and *TUBA1A* genes are summarized in Table II.

Patient with mutation in the *TUBA1A* gene (c.1160C≥T, p.A387V)

This patient is a female, aged 5 years, who is the only child of non-consanguineous parents. Her birthweight was 2570g (10th centile), length was 46cm (3rd centile), and head circumference was microcephalic (occipitofrontal circumference 31cm; below the 3rd centile). Her perinatal history was uneventful. The main developmental milestones appeared delayed (e.g. she walked unaided at 30mo). At 5 years, ophthalmological examination revealed alternating esotropia. Electroencephalography (EEG) showed irregular organization of the background activity and slow abnormalities in the central anterior regions.

At the neurological examination, microcephaly (occipitofrontal circumference 45cm, below the 3rd centile), clumsiness, mild ataxia of gait, and severe intellectual disability with absence of language were evident. Interpersonal skills and social interaction were poor. Mild facial dysmorphic features such as upslanting palpebral fissures and epicanthus were observed.

Brain MRI at age 5 showed pachygyria associated with a diffuse subcortical band heterotopia that spared the

Table I: Different malformations of cortical development categories. Classification scheme from Barkovich et al.[1]

Malformations of cortical development groups	No. of participants (*n*=79)	Categories	No. of participants classified in category	Subcategories	No. of participants classified in subcategory
Abnormal neuronal and glial proliferation or apoptosis	9	Severe congenital microcephaly associated with or without other brain abnormalities and different degrees of cognitive delay	6		
		Focal and multifocal cortical and subcortical dysgenesis	3		
Abnormal neuronal migration	27	Different patterns of periventricular heterotopias	18	Anterior predominant	13
				Posterior predominant	3
				Periventricular not-nodular	1
				Ribbon-like	1
		Abnormal transmantle migration	8	Anterior predominant	2
				Posterior predominant lissencephaly and subcortical band heterotopia	6
		Abnormal late radial or tangential transmantle migration	1	Subcortical heterotopia (other than band heterotopia or cortical infolding)	1
Abnormal post-migrational development	43	Polymicrogyria with schizencephaly	13		
		Polymicrogyria without clefts or calcifications	22	Generalized	5
				Bilateral perisylvian	4
				Frontal	3
				Parasagittal	3
				Unilateral perisylvian	3
				Hemispheric	2
				Parasagittal mesial occipital	1
				Posterior	1
		Complex malformation syndromes with polymicrogyria	5	Aicardi syndrome	3
				Polymicrogyria associated with other major brain dysmorphic features	2
		Focal cortical dysplasia	3		

frontobasal regions only. The heads of the caudate nuclei were fused with the putamen so that the anterior limbs of the internal capsules could not be identified bilaterally. The corpus callosum was thin and the anterior commissure was absent; the hippocampi showed a simplified pattern. The brainstem was highly dysmorphic with an abnormal transition between the medulla and a flattened pons, a thickened mesencephalon, and a thinned pontomesencephalic junction. The cerebellar vermis was mildly hypoplastic (Fig. 1a–d).

Patient with mutation in the *TUBB2B* gene (c.1080_1084del CCTGAinsACATCTTC)

This patient is a female, aged 31, who is the first daughter of non-consanguineous parents. Delivery at term was unremarkable. Her birthweight was 3400g (50–75th centile), length was 48cm (25th centile), and head circumference was below the 3rd centile (occipitofrontal circumference 32.5cm). Her perinatal history was uneventful. The main developmental milestones appeared severely delayed with absence of expressive language. A deflection of head growth curve was observed. Epilepsy was characterized by spasms (which started at 7mo) and a hypsarrhythmic pattern on EEG. Adrenocorticotropic hormone therapy achieved good seizure control. At 12 months, focal complex epileptic seizures relapsed. The patient was started on phenobarbital, which achieved partial seizure control. She has been seizure free since the age of 10 years. At the most recent evaluation (31y), EEG did not show any epileptiform activity. She displayed optic hypoplasia with optic atrophy (her left eye was more compromised than her right eye). At the neurological examination, marked microcephaly (occipitofrontal circumference 47.5cm, markedly below the 3rd centile), truncal hypotonia with no head control, symmetrical limb spasticity, and severe intellectual disability were evident. Language was absent. Severe thoracolumbar scoliosis was observed. Moreover, the patient showed poor social interaction and signs of self-harm. A 3T MRI examination showed severe microcephaly with a simplified gyral pattern, enlarged and dysmorphic lateral ventricles, a thinned corpus callosum, and hypoplastic temporal lobes. The anterior commissure was absent. A very small heterotopic nodule was evident along the wall of the right ventricle and an area of heterotopia was present in the white matter above. The basal ganglia and thalami were dysmorphic, characterized by a fusion between the caudate nuclei and putamen and by an abnormal and incomplete representation of both internal capsules. The hippocampi had a

Table II: Clinical and neuroradiological findings of published cases with mutations in the *TUBA1A* and *TUBB2B* genes

	TUBA1A gene[a]	*TUBB2B* gene
Nr of patients examined	42[6,11,12,14–19,27]; males=14; females=11;	19[12,13,20,21,23,29]; males=10; females=9
Microcephaly	18/42 (42.8%);	14/17 (82%; no data in 1/5[20])
Ocular findings	Strabismus: (1/3),[16] (2/8)[27];	Ptosis/CFEOM: 4[21,29]; strabismus: 2[13]
Intellectual disability	21/42 (50%); no data in (4/42): 3/8,[11] 1/5[27]	18/19 (100%; no data in one fetus)
Severe	(17/26): 2/4,[6] 5/8,[11] 1/2,[12] 1/1,[15] 1/3,[16] 1/1,[17] 1/1,[18] 1/1,[19] 4/5[27]	13/19[12,13,20,21,23]
Moderate	(3/9): 1/4[6], 1/2,[12]1/3[16]	2/19[23]
Mild	1/3[16]	3/19[29]
Epilepsy	16/42[6,11,12,14–19,27] (38%); no data in 3/8,[11]	11/19[12,13,20,21,23] (58%; no data in one fetus)
Cortex	41/42 (97.7%)	19/19 (100%)
Agyria–pachygyria	2/4,[6] 7/8,[11] 5/17,[14] 1/1,[18] 5/5[27b]	2/19 [12,23]
Lissencephaly	12/17,[14] 1/1,[15] 1/1,[17] 1/1[19]	
PMG	1/4,[6] 2/2,[12] 3/3[6]	17/19[12,13,20,21,23,29]
Corpus callosum	41/42 (97.7%)	16/19 (84%)
Hypoplasia	9[6,11,12,16,27]	5/19[13,23,29]
Agenesis	27[11,12,14,15,17,18,27]	
Dysmorphism	5[11,14]	
Anterior commissure	Data not reported	Data not reported
Basal ganglia	5/42 (12%)	17/19 (89.5%)
Dysmorphism	5[12,15–18]	17/19[12,13,20,21,23,29]
Hippocampi	23/42 (55%)	2/19 (11%)
Abnormal lamination	23[11,14,17,27]	2/19[20]
Ventricles	12/42 (28.6%)	10/19 (52.6%)
Enlarged	12[11,12,14,17]	8/19[12,13,21]
Brainstem	13/42 (31%)	15/19 (79%)
Dysmorphism		12/19[12,20,21,23]
Hypoplasia	13[6,12,14,19,27]	3/19[13]
Cerebellum	28/42 (66.6%)	16/19 (84%)
Hypoplasia	28[6,11,12,14,15,17–19,27]	13/19[12,13,20,21,23]
Abnormal foliation		3/19[29]
Optic nerves		
Optic nerve hypoplasia	(1/4)[6]	1[21]
Optic atrophy	(1/2)[12]	3[12,20]
Others	Absent septum pellucidum: 2[6,12]	PCSP: 3/19[12,23]

[a]No clinical data in 17 patients reported in Kumar et al.[14] [b]Associated subcortical band heterotopia in 2/5. NA, not applicable; CFEOM, congenital fibrosis of the extraocular muscles; PMG, polymicrogyria; PCSP, partial cavum septum pellucidum.

simplified appearance. A cortical–subcortical gliotic lesion of unknown origin was present in the right occipital lobe (the patient had a normal pre- and perinatal period and no significant trauma was reported). The cerebellar cortex in the upper paravermian region showed an irregular disposition of the folia; hypoplasia of the vermis was evident. Optic nerves were small (Fig. 1e–l).

DISCUSSION

The frequency of novel mutations found in the present study (3.8%, 3 out of 79) confirms the relevance of tubulin genes in determining MCDs, as found by other population studies.[12,14,16,20] The features of cortical maldevelopment enrich the spectrum of the MCDs tubulin-related disorders described so far: the patient carrying a mutation in the *TUBB2B* gene (Patient 2) showed areas of nodular heterotopia never before reported; the patients carrying a mutation in the *TUBA1A* gene presented with pachygyria associated with a diffuse subcortical band heterotopia which, among lissencephaly spectrum disorders, has so far been observed in only two cases, described by Morris-Rosendahl et al.[27] Additional defects of migration included malformed hippocampi and irregular disposition of the cerebellar folia in the paravermian region in Patient 2. A quite similar cerebellar cortical dysplasia has previously been associated with a *TUBA1A* gene mutation,[12,13] thus giving rise to new hypotheses on the role of the tubulin genes in development of the cerebellar cortex.

In addition to MCDs, MRI and tractography studies of patients with mutations in the tubulin genes (see Figs. 2 and 3) show several white matter brain malformations, raising the intriguing hypothesis that mutations in the tubulin genes superfamily cause primary generalized defects in axon guidance. As consistent features, these patients present with dysmorphisms of the pons (with an abnormal course of transverse pontine fibres, as shown by diffusion tensor imaging tractography) and basal ganglia (fusion between the caudate nucleus and putamen with absence of the anterior limb of the internal capsule), defects in commissural fibre tracts (anterior commissure and corpus callosum abnormalities/agenesis) and fornix and optic nerve hypoplasia/atrophy, confirming the key role of tubulin genes in axon tract formation as broadly described.[5,7,21,25,28,29] Intriguingly, defects of the anterior commissure have never been reported in the literature (see Table II), although they may have been underdiagnosed. In this case, we suggest it could be a new hallmark of tubulin disorders such as the basal ganglia dysmorphism

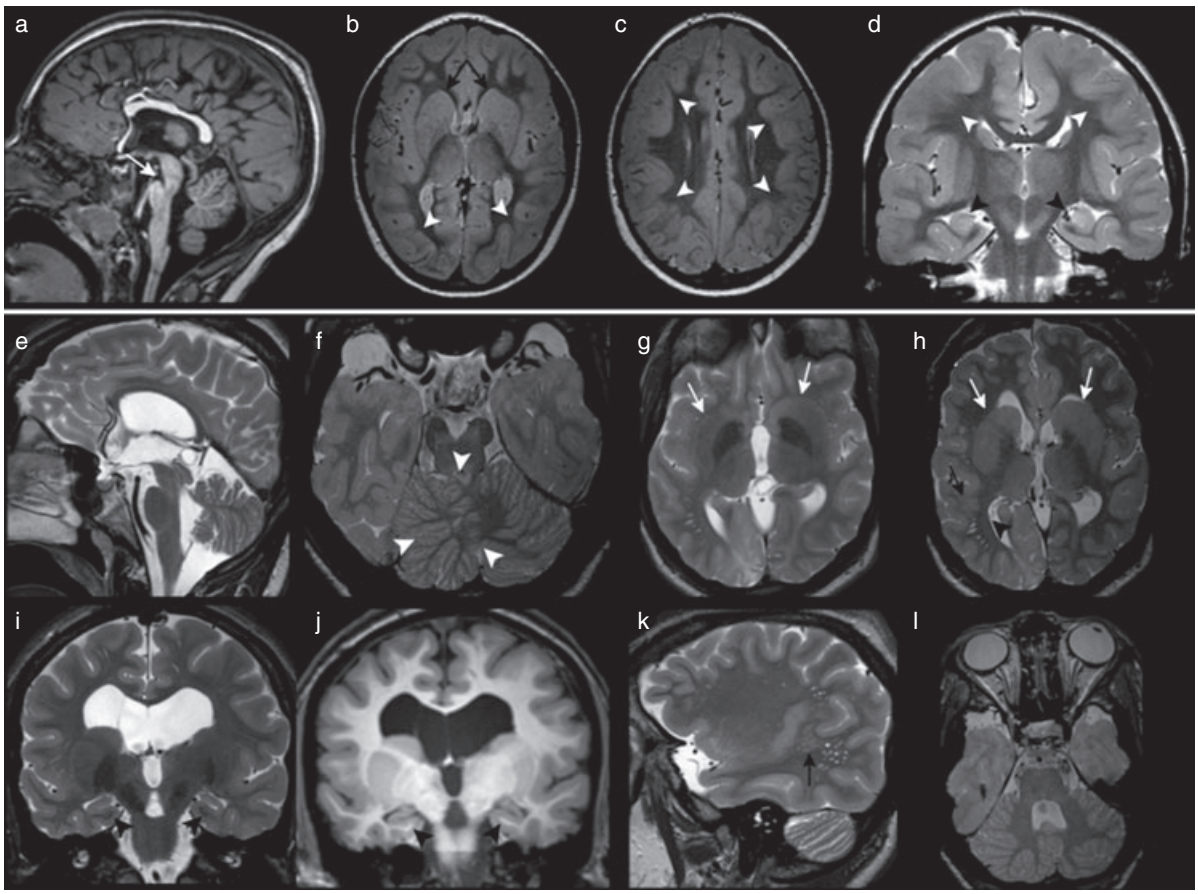

Figure 1: 3T MRI findings: sagittal (a) and coronal (j) T1-weighted; and sagittal (e, k), axial (b, c, f, g, h, l), and coronal (d, i) T2-weighted sections. Patient 1 (a–d, 5y) showed a pachygyric cortex with diffuse subcortical band heterotopia (white arrowheads in b, c and d), irregular basal ganglia with a fusion between caudate nuclei and putamina, and a simplified aspect of the hippocampi (black arrowheads in d). In (a) the corpus callosum appears thinned and truncated, the anterior commissure is absent, and the midbrain is highly dysmorphic (the white arrow points at a thinned pontomesencephalic junction). Patient 2 (e–l, 31y) showed several brain abnormalities on 3T MRI: the cerebellar vermis is hypoplasic and there is an irregular disposition of the cortical folia in the upper paravermian region (white arrowheads in f); the basal ganglia have an abnormal shape with a poor definition of the anterior limbs of the internal capsules (white arrows in g and h); the hippocampi show a simplified pattern (i and j); the optic nerves are thinned (l). A very small heterotopic periventricular nodule (black arrowhead in h) associated with an area of heterotopia in the white matter above (black arrows in h and k) is also evident. The lateral ventricles are enlarged, the corpus callosum is thin, and the anterior commissure is absent (e). An area of gliosis is evident in the right occipital lobe (g and h).

hypothesized by Amrom et al.[13] Moreover, as regards the malformation of the hippocampi, the role of the *TUBA1A* gene in hippocampal neurogenesis, as described by Keays et al.,[30] should be mentioned.[30] Whether the axon tract abnormalities are a primary or a secondary defect is still a matter of discussion. However, while this is under debate for the *TUBA1A* and *TUBB2B* mutations, axon guidance defects seem to be mainly associated with mutations in the *TUBB3* gene.[9,16,25] In this regard, a recent study provides new evidence that a mutation in the *TUBB2B* gene (E421K) can cause primary axon guidance defect by altering the kinesin localization and microtubule dynamics. Our findings support the hypothesis of primary axon guidance defects in *TUBB2B* and *TUBA1A* mutations alike.[29]

The putative pathogenic role of the mutations reported herein is more evident and more easily hypothesized in the case of the ins/del *TUBB2B* mutation. The complex rearrangement generates an abnormal protein lacking one residue and carrying three additional amino acids within the intermediate domain of the *TUBB2B* gene. This sequence change results in a longer β-strand, which may affect the interaction and the structural rearrangement of this domain with the N-terminus: an interaction physiologically occurring as a result of the hydrolysis of the Guanosine-5′-triphosphate (GTP) bound by the β-tubulin. The missense mutation identified in the *TUBA1A* gene, p.Ala387Val, falls within the C-terminus which is known to be highly conserved among α-tubulins owing to its functional role in GTP binding and in the motor proteins or microtubule-associated protein interaction.[5,10] The extremely high evolutionary conservation of the mutant residue and its

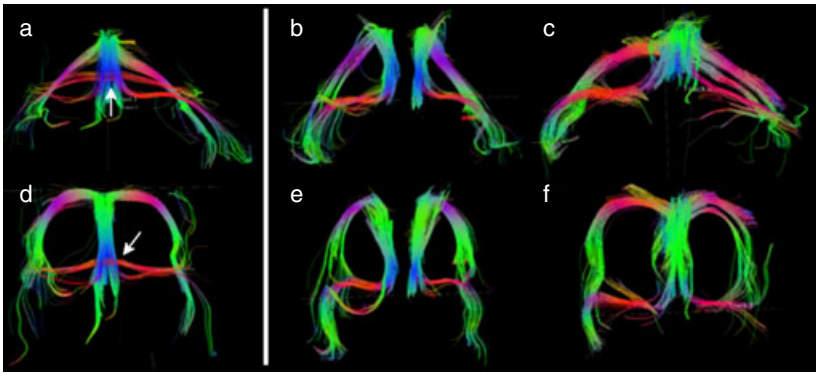

Figure 2: Track representations of fornix and anterior commissure. Frontal and superior views of fornix and anterior commissure (white arrows) in a typical individual (a and d) and in Patients 1 (b and e) and 2 (c and f) are shown. In the patients carrying mutations the red bundle representing the anterior commissure is absent. In Patient 1 fornices appear abnormally distant and in Patient 2 the tracts are thinner and have a more flattened course than normal.

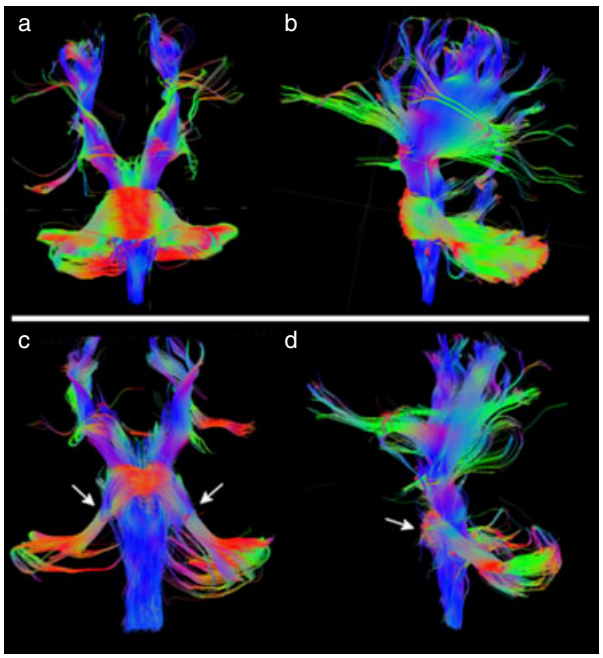

Figure 3: Track representations of pontine fibres and corticospinal tracts. Frontal and sagittal views of pontine transverse bundles and corticospinal tracts in a normal participant (a and b) and in Patient 1 (c and d). Compared with the typical individual, pontine transverse fibres in Patient 1 are markedly thinner and have a more slanting course (white arrows). The corticospinal tracts appear to be thinner, too, and less widely distributed in the cerebral hemispheres.

flanking regions, and its location in relative proximity to the α/β-tubulin interface, point towards a relevant functional role of the Ala387 residue. The p.Ala387Val substitution is located in an exposed α-helix at the C-terminus. Similar to the predicted effects of the substitution of a close residue, Arg-390,[15] the p.Ala387Val substitution may affect the interaction with microtubule-associated protein or motor proteins by modifying a relevant protein–protein interface.

CONCLUSION

The present study confirms that mutations in tubulin genes are responsible for complex brain malformations including a wide range of non-isolated MCDs and additional brain abnormalities involving the basal ganglia and anterior commissural structures. These should guide the selection of patients for the analysis of mutations in the *TUBB2B* and *TUBA1A* genes.[13] Unlike mutations in genes such as *DCX*, *FLNA*, or *Reelin*, each of which is associated with particular brain phenotypes,[1] mutations in different tubulin-encoding genes share a common complex pattern of brain malformations. Therefore, these genes should be tested when the presence of complex brain malformations characterized by abnormal commissural structures, dysmorphic basal ganglia, and hippocampi associated with MCDs is identified, as white matter involvement is a peculiar feature of tubulin-related disorders.

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SUPPORTING INFORMATION

The following additional material may be found online:

Figure S1: *TUBA1A* and *TUBB2B* mutations identified.

Data S1: Methods.

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