

CASE REPORT

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Case reports: novel *TUBG1* mutations with milder neurodevelopmental presentations



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Abstract

Background: Tubulinopathies result from mutations in tubulin genes, including *TUBG1*, responsible for cell microtubules, are characterized by brain development abnormalities, microcephaly, early-onset epilepsy, and motor impairment. Only eleven patients with *TUBG1* mutations have been previously described in literature to our knowledge. Here we present two new patients with novel de novo *TUBG1* mutations and review other cases in the literature.

Case presentations: Both patients have microcephaly and intellectual disability. Patient B further fits a more typical presentation, with well-controlled epilepsy and mild hypertonia, whereas Patient A's presentation is much milder without these other features.

Conclusion: This report expands the spectrum of *TUBG1* mutation manifestations, suggesting the possibility of less severe phenotypes for patients and families, and influencing genetic counselling strategies.

Keywords: Microcephaly, *TUBG1*, Tubulin, Malformations of cortical development, Intellectual disability, Epilepsy

Background

Mutations in the tubulin genes (e.g. *TUBA1A*, *TUBB2A*, *TUBA8*, *TUBB2B*, *TUBB3*, *TUBB5*, *TUBG1*) are associated with a range of brain malformations. Common tubulinopathic presentations include an array of lissencephalies, polymicrogyria-like cortical dysplasia, simplified gyral pattern, microlissencephaly, and a dysmorphic corpus callosum [1, 2]. Eleven patients with a *TUBG1* mutation have been described in the literature to our knowledge, and only limited clinical information is available for these patients. They are described with microcephaly, motor impairment, intellectual disability and epilepsy [1, 3, 4]. Here we report two further patients with novel *TUBG1* mutations and milder presentations.

Case presentations

These two patients are followed at BC Children's Hospital, a public academic tertiary pediatric referral centre, serving a population of nearly 5 million people.

Patient A is a 10-year old right-handed female born following a pregnancy complicated by antenatal microcephaly noted on fetal ultrasound. She is of Chinese descent and has no family history of consanguinity or

congenital anomalies. Her early development milestones were normal; she sat at 6 months, crawled at 8 months and walked at 12 months of age. Her mild intellectual disability was first apparent at the age of six years and she is now two years behind her peers academically, with no regression in development. She speaks two languages. She has an independent education plan. At birth her head circumference was not measured and by ten years of age the patient's head circumference was 46 cm (2 SD below the mean). She has some dysmorphic facial features including prominent ears relative to her microcephaly, a tented mouth, and bilateral 5th finger clinodactyly. Neurological examination was otherwise unremarkable. Magnetic resonance imaging (MRI) at nine years of age revealed microcephaly, posteriorly predominant simplified cortical gyri, and areas of band and nodular heterotopia (Fig. 1). No seizure-like activity has been described by the parents but a screening electroencephalogram (EEG) demonstrated occasional interictal sharp waves over the right central temporal areas in drowsiness and sleep, suggestive of a predisposition towards focal onset seizures, in addition to occasional, non-specific, generalized paroxysmal delta activity in sleep. Chromosomal microarray and biochemical screening for inborn errors of metabolism (as described elsewhere) were both unremarkable [5]. Clinical whole

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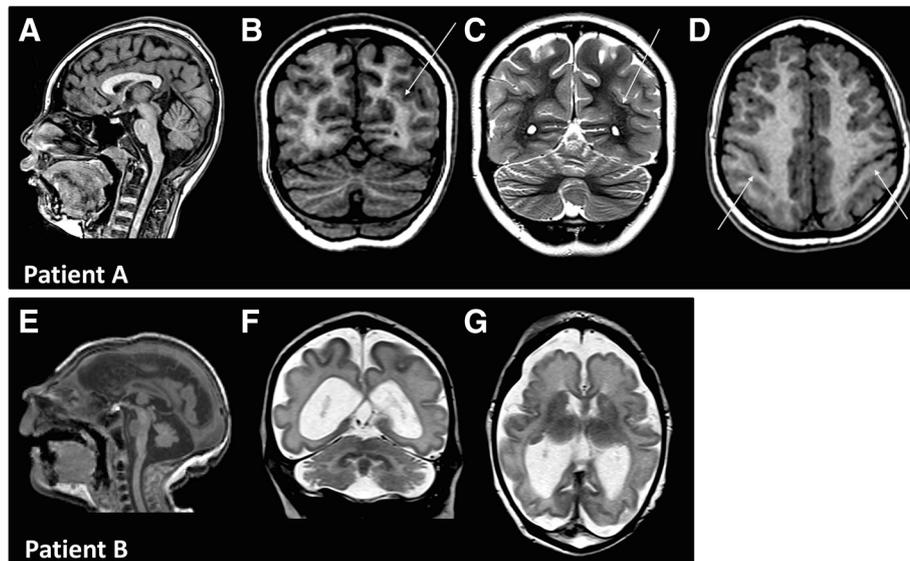


Fig. 1 MRI Findings of Patient A with *TUBG1* p.Asp68Asn mutation and Patient B with *TUBG1* p.Arg341Trp mutation. **a** Sagittal T1-weighted MPRAGE image demonstrates reduced craniofacial ratio in this child with microcephaly and posteriorly predominant simplified cortical gyri. The cerebellar vermis is normal. The corpus callosum appears slightly thick. **b** and **c** Coronal (**b**) T1 weighted MPRAGE and (**c**) Coronal T2 weighted fast spin echo images demonstrating bilateral parietal band heterotopia, more pronounced on the left (arrow). **d** Axial T1 weighted MPRAGE image shows bilateral blurring of the grey-white interface along the central sulci, in keeping with focal cortical dysplasia (arrows). **e** Sagittal T1-weighted image demonstrates microcephaly, intact corpus callosum and normal pituitary. The pons and cerebellar vermis are small for gestational age, but are notably less affected than the supratentorial brain. **f** and **g** Coronal (**f**) and axial (**g**) T2-weighted images demonstrating dilated lateral ventricles and simplified gyral pattern. In (**f**) there is prominence of the cerebellar folia but normal-appearing dentate nuclei. In (**g**) the basal ganglia and thalami are small and poorly defined, with absent myelination of the posterior limb of internal capsule

exome sequencing (Centogene AG, Rostock, Germany) revealed a novel, de novo *TUBG1* missense mutation (NM_001070.4: c.202G > A; p.Asp68Asn), using a trio approach (proband plus parents), chosen due to the multiple potential causative genes. The parents consented to this report.

Patient B is a 13-month old male born following a pregnancy complicated by fetal ultrasound findings of microcephaly (bitemporal narrowing), possible cavum septum pellucidum, enlarged ventricles, agenesis of the corpus callosum, and lissencephaly. He is of Caucasian descent with no family history of consanguinity or congenital anomalies, but a maternal history of simple febrile seizures and paternal history of asymptomatic Huntington's disease gene carriers. Head circumference at birth was below the 3rd percentile, (measured as 21 cm at 2 weeks of age), length 10-50th percentile, and weight 10th percentile. Up-slanting palpebral fissures were noted. Within the first hour of life, he experienced a seizure characterized by left-sided clonic activity with secondary bilateral synchrony and oxygen desaturation. This was managed with phenobarbital. The interictal EEG at this point showed diffuse suppression and excessive left central sharp waves, suggestive of cerebral dysfunction with some focality. A full septic workup was

completed and negative. MRI at the age of thirteen days demonstrated microcephaly, a small cerebellum, reduced number and complexity of the cerebral sulci and gyri with no cortical thickening or polymicrogyria, general paucity of cerebral white matter, dilated lateral ventricles, a normal third ventricle, small lentiform nuclei which were not well separated from the small thalami, and a grossly normal corpus callosum for age. There was normal myelination in the brainstem and cerebellum but there was lack of myelination in the posterior limbs of internal capsules. The patient had twenty-nine more seizures within the first forty-three days of life. These were twenty to forty seconds in length and generally associated with feeding. They involved a combination of apnea and cyanosis, lip smacking, head turning to the right, left eye deviation, arm flexion, leg extension, and post-ictal fatigue. At last follow-up, seizures were controlled with levetiracetam and topiramate. He has central hypothyroidism on thyroxine but otherwise normal pituitary function. He was also found to have small optic nerves, and a small secundum atrial septal defect shunting left to right. At 13 months, last follow up, he is not yet rolling or sitting independently, but has head control, brings his hands to the midline, is babbling and visually fixing and following. Neurological examination is notable for slightly low axial

tone and increased appendicular tone, with a head circumference of 36 cm (below 3rd percentile). Chromosomal microarray and biochemical screening for inborn errors of metabolism were both unremarkable [5]. Clinical whole exome sequencing (GeneDx, Gaithersburg, USA) revealed a novel, *de novo* *TUBG1* missense mutation (NM_001070.4: c1021C > T; p.Arg341Trp) using a trio approach. The parents consented to this report.

Discussion and conclusions

Tubulinopathies are characterized by a wide range of brain malformations, including lissencephaly, polymicrogyria and mildly simplified gyral patterning. The full phenotypic spectrum of tubulinopathies is however not yet fully known. All but one of the eleven reviewed cases of *TUBG1* mutations involved epilepsy, majority of which were refractory and early-onset in nature. Of the seven patients with birth head circumference measurements, six had microcephaly (<2 standard deviations (SD) below the mean) and four of the six had severe microcephaly (<3 SD below the mean). Motor dysfunction was present in all but one patient out of the nine with the available data, ranging from delayed motor development to spastic tetraplegia. All patients suffered from intellectual disability ranging from moderate to severe [1, 3, 4].

Patient A represents the least severe manifestation of *TUBG1* mutation reported so far, having microcephaly, brain malformations, mild facial dysmorphism, and mild intellectual disability, but no motor impairment or epilepsy. Patient B had a more typical presentation sharing features with the more severe phenotype: microcephaly, brain malformations, global development delay and early-onset epilepsy (though easily controlled). Other neurological, clinical, and genetic phenotypes are compared in summary in Table 1.

Whereas tubulin gene mutations often follow an autosomal dominant mode of inheritance [2], *TUBG1* mutations are almost entirely *de novo* [1, 3, 4]. Furthermore, tubulinopathies encompass a range of phenotypes including extreme lissencephaly, severe cerebellar hypoplasia, and varying cortical thickness [6, 7]. Despite overlap between the brain phenotypes of different tubulinopathies, certain phenotypes are associated with specific tubulin genes [8]. *TUBG1* patients, are characterized by pachygyria/agyria that is most intense in the parieto-occipital regions with a posterior to anterior gradient as well as enlarged lateral ventricles and reduced white matter volume [3, 4]. Lastly, unlike other tubulinopathies, majority of *TUBG1* mutation patients have a normal cerebellum, basal ganglia, and brainstem [1, 4]. Patient A coincides with the known *TUBG1* presentation involving posteriorly-predominant pachygyria and a normal cerebellum, basal ganglia, and brainstem.

However, this patient diverges from the known *TUBG1* mutation phenotype due to the presence of band and nodular heterotopia, normal white matter volume, and normal lateral ventricle size. Patient B is similar to known *TUBG1* mutation in having pachygyria (although not posteriorly-predominant), dilated lateral ventricles, reduced white matter, normal cerebellum, and brainstem. Unlike typical *TUBG1* mutation phenotype, this patient has a small cerebellum. In consideration of the microcephaly spectrum, radiologically, Patient B may correspond to a label of congenital microcephaly with a simplified gyral pattern [9].

TUBG1 encodes for γ -tubulin, highly expressed in the developing fetal brain as a component of centrosomes. It plays an integral role in microtubule nucleation, thereby affecting microtubule-dependent mitosis and brain development [3, 4]. Poirier et al. introduced mutations in the γ -tubulin gene (*tub4*) in *S. cerevisiae* which interfered with microtubule nucleation. Suppression of *TUBG1* in utero of mice also arrested neuronal migration [3]. Depending on the mutation locus, the different *TUBG1* mutations are thought to have an effect on γ -tubulin structure or function. It is suspected that *TUBG1* mutations affect neuronal migration hence MRI findings showing pachygyria/agyria. This is in comparison to other tubulinopathies which are more associated with polymicrogyria or dysgyria [4].

We suspect the *TUBG1* mutations found to be likely pathogenic. Both missense mutations described in this paper were *de-novo* resulting in substitutions in highly conserved amino acids which are predicted to be damaging by multiple *in-silico* algorithms, including SIFT, PolyPhen-2, MutationTaster2. Neither mutation has been reported in public databases (gnomAD Browser), and two likely pathogenic entries are present in ClinVar for the Arg341 residue (Patient B). The p.Asp68Asn mutation (Patient A) affects a highly conserved residue in all 20 human tubulin proteins and their ancestral homologs. The Asp68 residue is located in the GTP-binding pocket and together with the Glu72 coordinates the Mg²⁺ ion that interacts with the GTP γ -phosphate [10, 11] (Fig. 2). The p.Arg341Trp mutation (Patient B) substitutes a positively-charged arginine with an aromatic amino-acid, within the Tubulin/FtsZ 2-layer sandwich (or C-terminal) domain (Fig. 2). The Arg341 residue is part of a stretch of polar amino acids (Arg339, Arg341, Glu342, Arg343 and Lys344) at the interface of γ - γ tubulin dimer and plays a critical role in the assembly of the γ tubulin homodimer by forming multiple H-bonds with residue Asp252 on the opposite chain [12].

We present here two patients each with a novel, *de novo* *TUBG1* mutation with common features of microcephaly and intellectual disability but lacking both spastic tetraplegia and more severe refractory epilepsy. The

Table 1 Clinical, imaging, and genetic features of patients with *TUBG1* mutations (Continued)

ID	Patient A	Patient B	Patient 1 ^{1,3}	Patient 2 ^{1,3}	Patient 3 ^{1,3}	Patient 4 ⁴	Patient 5 ⁴	Patient 6 ⁴	Patient 7 ⁴	Patient 8 ⁴	Patient 9 ⁴	Patient 10 ⁴	Patient 11 ⁴
Corpus callosum (MRI)	band heterotopia, nodular heterotopia		pachygyria/ agyria (posterior agyria, frontal pachygyria), thick cortex	pachygyria/ agyria (posterior agyria, frontal pachygyria), thick cortex	posterior subcortical band heterotopia	(posterior frontal lobe and parieto-occipital cortex)	Thin	(mild over frontal lobes, moderate over posterior lobes), cortex 10-13 mm thick	sparse cells over occipital lobes, cortex 13-15 mm thick	(mild over frontal lobe, moderate over temporal and occipital lobes), cortex 6-13 mm thick	(mild over frontal lobe, moderate over temporal and occipital lobes), cortex > 15 mm thick	(almost normal over frontal lobes, moderate over pachygyria)	(mild over frontal lobe, moderate over temporal and occipital lobe, deep parietal lobe infolding)
Other MRI Findings		Normal	Thin	Thick, dysmorphic	Thick, dysmorphic	Normal	Thin	Normal	Normal	Normal	Thin	Normal	Thin
		General paucity of white matter, small cerebellum, dilated lateral ventricles, small lentiform nuclei, small thalami, posterior limbs of internal capsule lacking myelination	Mildly enlarged lateral ventricles, mildly reduced white matter	Mildly enlarged lateral ventricles, severely reduced white matter	-	Enlarged perivascular spaces, enlarged posterior horns of lateral ventricles, hippocampal malrotation	Severely enlarged lateral ventricles, severely reduced white matter, dysplastic basal ganglia, hypoplastic brainstem, hypoplastic vermis	Mildly enlarged lateral ventricles, mildly reduced white matter	Mildly enlarged lateral ventricles, mildly reduced white matter	Enlarged posterior horns of lateral ventricles	Mildly enlarged lateral ventricles	Mildly enlarged posterior horns of lateral ventricles, mildly reduced white matter	Mildly enlarged lateral ventricles, mildly reduced white matter, dysplastic basal ganglia

F female, M male, y years, mo months, SD standard deviation, ID intellectual disability, - absent, N/A not available

Consent for publication

Although no personally identifiable information is presented in this study, written informed consent for publication was obtained from the parents/legal guardians of the two children (under 18) reported in this study.

Competing interests

The authors of this manuscript have no financial or non-financial competing interests to declare.

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References

1. Bahi-Buisson N, Poirier K, Fourniol F, et al. The wide spectrum of tubulinopathies: what are the key features for the diagnosis? *Brain*. 2014; 137(6):1676–700. <https://doi.org/10.1093/brain/awu082>.
2. Bahi-Buisson N, Cavallin M. Tubulinopathies Overview. *Ncbi.nlm.nih.gov*. <https://www.ncbi.nlm.nih.gov/books/NBK350554>. Published 2018. Accessed 14 Sept 2018.
3. Poirier K, Lebrun N, Broix L, et al. Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat Genet*. 2013;45(6):639–47. <https://doi.org/10.1038/ng.2613>.
4. Brock S, Stouffs K, Scalais E, et al. Tubulinopathies continued: refining the phenotypic spectrum associated with variants in TUBG1. *Eur J Hum Genet*. 2018;26(8):1132–42. <https://doi.org/10.1038/s41431-018-0146-y>.
5. van Karnebeek CDM, Stockler-Ipsiroglu S. Early identification of treatable inborn errors of metabolism in children with intellectual disability: the treatable intellectual disability endeavor protocol in British Columbia. *Paediatric Child Health*. 2014;19(9):469–71.
6. Parrini E, Conti V, Dobyms W, Guerrini R. Genetic basis of brain malformations. *Mol Syndromol*. 2016;7(4):220–33. <https://doi.org/10.1159/000448639>.
7. Desikan R, Barkovich A. Malformations of cortical development. *Ann Neurol*. 2016;80(6):797–810. <https://doi.org/10.1002/ana.24793>.
8. Romaniello, et al. Tubulin genes and malformations of cortical development. *European Journal of Medical Genetics*. 2018;61(12):744–54. <https://doi.org/10.1016/j.ejmg.2018.07.012>.
9. Adachi Y, Poduri A, Kawaguchi A, et al. Congenital microcephaly with a simplified Gyral pattern: associated findings and their significance. *Am J Neuroradiol*. 2011;32(6):1123–9. <https://doi.org/10.3174/ajnr.a2440>.
10. Aldaz H, Rice L, Stearns T, Agard D. Insights into microtubule nucleation from the crystal structure of human γ -tubulin. *Nature*. 2005;435(7041):523–7. <https://doi.org/10.1038/nature03586>.
11. Gombos L, Neuner A, Berynskyy M, et al. GTP regulates the microtubule nucleation activity of γ -tubulin. *Nat Cell Biol*. 2013;15(11):1317–27. <https://doi.org/10.1038/ncb2863>.
12. Suri C, Hendrickson T, Joshi H, Naik P. Molecular insight into γ - γ tubulin lateral interactions within the γ -tubulin ring complex (γ -TuRC). *J Comput Aided Mol Des*. 2014;28(9):961–72. <https://doi.org/10.1007/s10822-014-9779-2>.
13. Rice L, Montabana E, Agard D. The lattice as allosteric effector: structural studies of $\alpha\beta$ - and γ -tubulin clarify the role of GTP in microtubule assembly. *Proc Natl Acad Sci*. 2008;105(14):5378–83. <https://doi.org/10.1073/pnas.0801155105>.

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