Tubulinopathies continued: refining the phenotypic spectrum associated with variants in TUBG1

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Abstract
Tubulinopathies are a heterogeneous group of conditions with a wide spectrum of clinical severity resulting from variants in genes of the tubulin superfamily. Variants in TUBG1 have been described in three patients with posterior predominant pachygyria and microcephaly. We here report eight additional patients with four novel heterozygous variants in TUBG1 identified by next-generation sequencing (NGS) analysis. All had severe motor and cognitive impairment and all except one developed seizures in early life. The core imaging features included a pachygyric cortex with posterior to anterior gradient, enlarged lateral ventricles most pronounced over the posterior horns, and variable degrees of reduced white matter volume. Basal ganglia, corpus callosum, brainstem, and cerebellum were often normal, in contrast to patients with variants in other tubulin genes where these structures are frequently malformed. The imaging phenotype associated with variants in TUBG1 is therefore more in line with the phenotype resulting from variants in LIS1 (a.k.a. PAFAH1B1). This difference may, at least in part, be explained by gamma-tubulin’s physiological function in microtubule nucleation, which differs from that of alpha and beta-tubulin.

Introduction
The gamma-tubulin protein (TUBG1) was first described by Oakley and Oakley [1], and the encoding TUBG1 gene has later been mapped to chromosome 17q2 [2–4]. TUBG1 shares 94.6% of nucleotides and 97.3% of amino acids with its paralog TUBG2, but despite this high degree of similarity they appear not to be functionally synonymous. Both TUBG1 and TUBG2 are highly expressed in the developing and mature human brain, including the cerebral cortex, cerebellum, thalamus, and hippocampus, with TUBG1 being expressed more abundantly than TUBG2 [5]. Variants in genes belonging to the tubulin superfamily, including TUBA1A, TUBB2A, TUBB2B, TUBB3, TUBB, and TUBG1, have been associated with a spectrum of

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cortical malformations through disruption of normal microtubule interactions, which are involved in neuronal cell proliferation, migration, and differentiation, as well as axon growth and guidance [6–15]. Microtubule nucleation precedes the formation of bipolar spindles and separation of chromosomes in mitosis, steps that are necessary for the progression of the cell cycle [16, 17]. Unlike alpha-tubulin and beta-tubulin, gamma-tubulin is not incorporated in the microtubule lattice but is required for the polymerization of the alpha-tubulin and beta-tubulin proteins. Therefore, gamma-tubulin localizes to the centrosome during interphase. This process is mediated by protein kinases [18]. Two gamma-tubulin proteins associate with gamma complex proteins 2 and 3 (GCP2, GCP3) to form a gamma-tubulin small complex (γTuSC). Binding of GCP4, GCP5, and GCP6 to several copies of γTuSC results in the formation of a γ-tubulin ring complex (γTuRC). γTuRC links microtubules to the spindle pole during mitosis [19]. Insufficient proliferation due to defective microtubule function can ultimately lead to microcephaly [8]. Defects in neuronal proliferation and migration are linked to the role microtubules play in cell shape and orientation [20].

Protein structures within the tubulin superfamily show a high degree of similarity. However, the phenotypic differences associated with variants in the various tubulin isotypes support the hypothesis that each tubulin has a distinctive function [21].

An alanine-scanning mutagenesis screen of human gamma-tubulin in *S. pombe* showed that all deleterious variants in the *TUBG1* gene were found in residues predicted to be located at the surface, some in positions to interact with alpha and/or beta-tubulin at the microtubule lattice. The localization of these variants might therefore indicate domains within the protein that are responsible for gamma-tubulin’s individual function [22, 23].

To date, three unrelated patients with de novo variants in *TUBG1* have been reported [14]. Two had microcephaly and bilateral symmetric pachgyria with a posterior to anterior gradient on imaging. They suffered from spastic quadriaparesis and were bedridden. The third patient presented with a milder phenotype with normal head circumference, mild intellectual disability, and posterior pachgyria. The corpus callosum was malformed in all three patients, whereas the basal ganglia, the cerebellum, and the brainstem were spared. All three patients presented with seizures in early life.

We report the identification of eight additional patients from seven families with four novel heterozygous variants in *TUBG1*, contributing to a further delineation of the associated clinical and imaging phenotype.

### Methods

#### Patient samples

Patients were recruited through the international research network of the authors. The study was approved by the Institutional Review Board of the UZ Brussel (B.U.N. 143201214360), the Institutional Review Board at Seattle Children’s Hospital, and the Pediatric Ethics Committee of the Tuscany Region. Informed consent was obtained from all families prior to genetic studies. Clinical data were collected through clinical examination and review of medical records. All imaging data were reviewed by AJ, NDD, and WBD.

Blood samples for DNA preparation and genetic investigation were obtained with informed consent from patients and parents. DNA was extracted using standard protocols.

For patients 1 and 2, variant analysis was performed using gene panel analysis. This analysis was performed at the Center of Medical Genetics, UZ Brussel in collaboration with the Brussels Interuniversity Genomics High Throughput core (BRIGHT core) according to standard procedures (see [http://www.brightcore.be/]). Raw data are quality controlled by use of FastQC (v0.10.1) and mapped to the human reference genome with BWA 0.7.10. Mapping qualities are assessed via overall coverage analysis by an in-house designed script. The mapped reads are processed using the GATK 2.7.2 (Genome Analysis Toolkit) pipeline (IndelRealigner, BaseRecalibrator, HaplotypeCaller) and the detected variants are annotated by Annovar or Alamut Batch.

Patients 3, 4, and 7 were studied using targeted panel sequencing with Single-Molecule Molecular Inversion Probes as previously described (PMID: 27773430) [24]. For patients 5 and 6 whole-exome sequencing (WES) was done at the Broad Institute Genomic Services using Agilent SureSelect enrichment kit with subsequent sequencing of the libraries on a HiSeq 2000 (Ilumina, San Diego, CA, USA). Sequence reads were aligned to the human genome (hg19) using BWA software or the CLC Biomedical Genomics workbench. Downstream processing was done with the Genome Analysis Toolkit, SAMtools, and Picard Tools. Single-nucleotide variants and indels were subsequently called by GATK Unified Genotyper (PMID:21478889) [25] and a variant quality score of ≥10 and were annotated using SeattleSeq SNP annotation and Annovar. Variants were then filtered using standard hard-filtering parameters (PMID:21478889) [25]. Specifically, only variants with a quality score of ≥30, sequencing depth of ≥10, quality/depth ratio of ≥5, length of homopolymer run of ≤5.0, and allelic balance of ≤0.80 were considered for downstream analysis.
For patient 8, WES was performed at the French National Centre for Genotyping (Evry, France). Library preparation, exome enrichment, WES, and analysis of variants were performed as previously described [26]. Exome sequencing quality data were homogeneous with an average mean depth higher than 100×. Coverage depth greater than 15× and 5× was obtained for about 97 and 99% of the target, respectively. We analyzed variants affecting coding regions and essential splice sites and excluded all variants with frequencies higher than 1% in multiple genome databases including the Single-Nucleotide Polymorphism Database, 1000 Genomes, the National Heart, Lung, and Blood Institute Exome Variant Server, the Exome Aggregation Consortium (ExAC), and a local Paris Descartes Bioinformatics platform database. The c.776C>T p.(Ser259Leu) variant was confirmed by Sanger sequencing and shown to be de novo. The reference sequence used was NM_001070.4 with systematic numbering of the exons (1–11); or as described in NG_033886.1. All variants have been submitted to https://databases.lovd.nl/shared/genes/TUBG1 (patient ID 00134040-00134047).

Discussion

Tubulinopathies have been characterized by a broad range of cortical malformations associated with hypoplasia or agenesis of the corpus callosum, dysmorphic basal ganglia, and hypoplasia of the brainstem, cerebellar vermis, and/or hemispheres [6, 9, 11, 27]. In some patients, the cerebellar dysgenesis and/or dysplastic basal ganglia may be more prominent than the cortical malformations [28].

Poirier et al. [14] initially described the phenotype associated with variants in TUBG1 as similar to that associated with variants in LIS1 (a.k.a. PAFAH1B1); an observation that has recently been integrated in the classification of lissencephalies proposed by DiDonato et al. [11]. A later report on the same patients mentioned that the two individuals with microcephaly and severe pachygyria resembled individuals with lissencephaly carrying the p.(Arg402Cys) substitution in TUBA1A [6].

In the current series including eight additional patients with variants in TUBG1, the most common imaging phenotype consists of partial or diffuse pachygyria with a posterior to anterior gradient, similar to the phenotype associated with variants in LIS1, DYNC1H1, or KIF5. However, the most severe end of the TUBG1-related spectrum also includes diffuse agryria as illustrated by patient 2, similar to the phenotype associated with some variants in LIS1 or the c.1205G>A, p.(Arg402His) variant in TUBA1A. The cortical malformation in patients with variants in TUBG1 therefore clearly stands out from that of tubulin-related dysgyria, which can be seen in patients with variants in TUBB2B, TUBB3, TUBB, and most variants in TUBA1A [11, 29].

In this study, associated brain abnormalities appear to be less frequent compared to other tubulinopathies. Except for two patients, all patients described so far had normal basal ganglia, which is usually considered a key feature for tubulinopathies and has been observed in 75% of patients...
| Clinical features | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 (LIS-TUB-027) | Patient 10 (LIS-TUB-028) | Patient 11 (LIS-TUB-029) |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------------------------|--------------------------|--------------------------|
| Reference         | This report | This report | This report | This report | This report | This report | This report | This report | Poirier et al. [14]      | Poirier et al. [14]      | Poirier et al. [14]      |
| **Sex**           | M         | F         | F         | F         | F         | M         | M         | F         | M                       | M                       | M                       |
| **Nucleotide sequence variation** | c.63C>A   | c.985G>T  | c.776C>T  | c.776C>T  | c.776C>T  | c.769A>T  | c.776C>T  | c.991A>C  | c.1160T>C               | c.275A>G               |
| **Protein sequence variation** | p.(Phe21Leu) | p.(Asp329Tyr) | p.(Ser259Leu) | p.(Ser259Leu) | p.(Ser259Leu) | p.(Ile257Phe) | p.(Ser259Leu) | p.(Thr331Pro) | p.(Leu387Pro) | p.(Tyr92Cys) |
| **Mode of inheritance** | de novo | de novo | de novo | de novo | de novo | de novo | de novo | de novo | de novo | de novo |
| **Age at examination** | 33y | 21y | 19mo | 14y | 11y 6mo | 9y 6mo | 15y | 18mo | 31y | 21y | 18mo |
| **Head circumference (SD)** | 57 cm (−3.6SD) | 47.5 cm at 6y 6mo (−3.3SD) | 47.5 cm at 6y 6mo (−3.3SD) | n/a | n/a | n/a | n/a | n/a | 51.3 cm at 13y (<−2.5SD) | Normal | <−5.5SD |
| **Dysmorphic features** | No | No pictures | No pictures | No pictures | No pictures | No pictures | No pictures | No pictures | No pictures | No pictures |
| **Congenital anomalies** | No | No | No | No | No | No | No | No | Cataract | n/a |
| **Intellectual disability** | Severe | Severe | n/a | n/a | Moderate | Moderate | Moderate | Moderate (FS IQ-score 44) | Severe | Moderate ID |
| **Motor impairment** | Spastic quadriplegia; walks with support | Spastic quadriplegia | Delayed motor development | Unsteady gait | Spastic diplegia | n/a | Delayed motor development | Moderate CP | Spastic quadriplegia | Spastic quadriplegia |
| **Speech and language development** | No speech, only sounds | Non-verbal | Delayed | Non-verbal | Speaks 50 words | Non-verbal | Speaks 5–6 word sentences | Non-verbal | n/a | n/a |
| **Other** | Assisted feeding | Gastrostomy | – | – | Drooling | – | – | – | – | – |
| **Epilepsy** | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Age at seizure onset** | 36m | n/a | 6m | 4m | n/a | 3y 11mo | 5m | n/a | n/a | n/a |
| **Seizure type** | Tonic–atonic–myoclonic partial complex: versive seizure, myoclonic | Tonic-clonic | Generalized tonic-clonic | n/a | n/a | Focal, versive | Early onset epilepsy | Early onset epilepsy | Infantile spasms |
| **Refractory** | n/a | Yes | – | n/a | n/a | n/a | n/a | No | Yes | Yes |

*Table 1: Clinical and imaging features associated with variants in TUBG1*
## Table 1 (continued)

| Clinical features | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 (LIS-TUB-027) | Patient 10 (LIS-TUB-028) | Patient 11 (LIS-TUB-029) |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------------------------|--------------------------|--------------------------|
| **Imaging features** |           |           |           |           |           |           |           |           |                         |                          |                          |
| Age at MRI | 36y | 11y | 1y 6mo | 12mo | 13y 7mo | 2mo | 6y | 9y | n/a | n/a | n/a | n/a |
| Gyral pattern | Pachygyria over the posterior frontal lobe and parieto-occipital cortex | Agyria, diffuse | Pachygyria diffuse, mild over frontal lobes, moderate posterior, cortex 10–13 mm | Pachygyria diffuse, mild over frontal lobes and moderate over temporal and occipital lobes, cortex 6–13 mm | Pachygyria, nearly normal cortex over frontal lobes, pachygyria over parietal and occipital lobes, cortex >15 mm | Pachygyria diffuse, mild over frontal lobes, moderate over temporal and occipital lobes, bilateral deep infolding parietal | Pachygyria diffuse, mild over frontal lobe, and moderate over temporal and occipital lobes | Pachygyria, nearly normal cortex over frontal lobes, pachygyria over parietal and occipital lobes | Pachygyria, nearly normal cortex over frontal lobes, pachygyria over parietal and occipital lobes | Pachygyria, nearly normal cortex over frontal lobes, pachygyria over parietal and occipital lobes | Pachygyria, nearly normal cortex over frontal lobes, pachygyria over parietal and occipital lobes | Pachygyria, nearly normal cortex over frontal lobes, pachygyria over parietal and occipital lobes |
| Gradient | P>A | P>A | P>A | P>A | P>A | P>A | P>A | P>A | P>A | P>A | P>A | P>A |
| White matter | Enlarged perivascular spaces | Severely reduced | Mildly reduced | Normal | Mildly reduced | Normal | Mildly reduced | Normal | Mildly reduced | Normal | Mildly reduced | Seveley reduced |
| Lateral ventricles | Enlarged posterior horns | Severely enlarged | Mildly enlarged | Mildly enlarged | Mildly enlarged | Mildly enlarged | Mildly enlarged | Normal | Mildly enlarged | Normal | Mildly enlarged |
| Corpus callosum | Normal | Thin | Normal | Normal | Normal | Thin | Normal | Thin | Dysmorphic, thick | Thin | Dysmorphic, thick |
| Basal ganglia | Normal | Dysplastic | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Hippocampus | Malrotation | n/a | Normal | Normal | Normal | Normal | Normal | Normal | Normal | n/a | n/a | n/a |
| Brainstem | Normal | Hydroplasia | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Cerebellum | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Cortex | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| White matter | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Vermis | Normal | Hydroplasia | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |

RefSeq NM_001070.4A anterior, CP cerebral palsy, F female, FS IQ full-scale IQ, ID intellectual disability, M male, mo months, n/a not available, P posterior, SD standard deviation, y years, – absent
Additionally, the brainstem and cerebellum were spared in most patients with TUBG1 variants, and if malformations in either of these two structures were present, they were usually subtle. This is in contrast to the high prevalence of 78.7% of cerebellar hypoplasia in individuals with variants in other tubulin genes [6, 28]. Polymicrogyria
or polymicrogyria-like cortical dysplasia and a simplified gyral pattern, which is common in TUBB2B and TUBB3 variants, have not been described in patients with TUBG1 variants so far. The observation that TUBG1 causes undermigration leading to pachgyria or agyria can possibly be explained by a negative impact on cell morphology in patients carrying a TUBG1 variant, while variants in other tubulin genes are more often associated with dysgyria or overmigration presenting as polymicrogyria which suggests defective radial glial guidance of immature neurons [8].

Thus, the observation that the imaging phenotype associated with variants in TUBG1 differs from that associated with variants in other tubulin genes is in line with the function of TUBG1 in nervous system development and its stronger involvement in neuronal migration than in, for example, axon growth and orientation, which appears to be more prominently affected by variants in TUBB3 [21, 30, 31]. This hypothesis is also supported by the disturbed neuronal migration observed in an in utero RNA interference assay by Poirier et al. [14]. Most patients with variants in TUBG1 also exhibit microcephaly,

**Fig. 2** Imaging characteristics patients 5–8. Brain MRI of patient 5 at age 13 years. Sagittal planes through the midline (a) show the absence of malformations of the corpus callosum, brainstem, and cerebellum. Axial T1-weighted images (b) and T2-weighted images (c) show pachgyria with a posterior to anterior gradient and enlarged posterior horns of the lateral ventricles. Brain MRI of patient 6 at age 2 months. Sagittal planes (d) show a thin corpus callosum, absence of hypoplasia of the brainstem, or the cerebellar vermis. Axial T2-weighted images (e, f) show diffuse pachgyria with a posterior to anterior gradient and enlarged lateral ventricles. Brain MRI of patient 7 at age 1 year 6 years. Sagittal images (g) show a normal appearance of corpus callosum, brainstem, and the cerebellum. Axial T2-weighted images (h, i) show pachgyria with a posterior to anterior gradient with almost normal frontal lobes, enlarged posterior horns of the lateral ventricles, and reduced white matter. Brain MRI of patient 8 at age 9 years. Sagittal images (j) show hypoplasia of the corpus callosum. Axial T2-weighted images (k, l) show pachgyria with a posterior to anterior gradient and important involvement of the temporal lobes, bilateral parietal infoldings, dysplastic basal ganglia, enlarged lateral ventricles, and reduced white matter.
indicating a major role of TUBG1 in neuronal cell proliferation.

It has been shown that tubulin isotypes have individual functions, expression levels, and distribution among different cell types, which led to the assumption that subtle phenotypic differences could exist. As the exact mechanism and the extent of how a variant alters the formation of functional tubulin heterodimers, GTP binding, longitudinal and lateral protofilament interactions, and microtubule interactions with microtubule-associated proteins remains widely unknown, so far, few conclusions can be drawn about these distinctive features [15, 21, 32, 33]. Nevertheless, the differences in physiological function of the TUBG1 protein as a scaffold in the formation of microtubules on the one hand and alpha-tubulin and beta-tubulin dimers as components of the microtubule on the other hand have been well established, and can give a possible explanation for the different phenotypic presentation on imaging [15].

The correlation between phenotype and genotype could further be determined by the exact location of the variant within the gene. This has been observed in the case of the recurrent c.1205G>A, p.(Arg402His) variant in TUBA1A causing classic lissencephaly, and the c.790C>T, p.(Arg264Cys) variant in the same gene associated with central pachygyria [6, 11, 34, 35]. Recurrent TUBB2B and TUBB3 variants have also been described to result in homogeneous phenotypes [15, 30, 36, 37]. This has also been observed in our study as the five patients with the c.776C>T, p.(Ser259Leu) variant shared a similar phenotype including mild frontal and moderate posterior pachygyria with no or subtle malformations of the corpus callosum, brainstem, and cerebellum. Additionally, patient 7 in our study carried a c.769A>T, p.(Ile257Phe) substitution which is only two amino acids upstream of the c.776C>T, p.(Ser259Leu) recurrent variant, and is associated with a similar phenotype. These variants are located at the borderline of the tubulin sandwich/C-terminal domain.

Interestingly, the c.985G>T, p.(Asp329Tyr) variant in patient 2, which was associated with the most severe phenotype in our series is located two amino acids upstream of the c.991A>C, p.(Thr331Pro) variant identified in a patient with posterior pachygyria and a moderate phenotype described by Poirier et al. [14]. These variants are also located in the tubulin sandwich/C-terminal domain. At this moment, it remains hard to predict the phenotype based on the position of the detected variants. However, c.985G>T, p.(Asp329Tyr) is located at the surface of the TUBG1 protein. In line with findings of the alanine-scanning mutagenesis, variants in surface proteins might have a more severe effect [22]. The majority of the variants are located in the 2-layered sandwich domain of the TUBG1 protein, which is probably involved in the formation of dimers (Fig. 3). Consequently, these variants are expected to interfere with the microtubule formation and have a dominant-negative effect on the function of TUBG1.

So far, no truncating variants have been described in patients with TUBG1 variants. Whether truncations are likely to have either more severe phenotypes or no associated phenotype remains unclear. However, the number of

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**Table 2** Overview of identified variants (RefSeq: NM_001070.4) and PolyPhen, SIFT, and MutationTaster scores

| Patient ID  | DNA | Protein | Exon | MAF | ExAC | PolyPhen2 | SIFT | Mutation-Taster | Align GVGD |
|------------|-----|---------|------|-----|------|-----------|------|----------------|-----------|
| 1          | c.63C>A | p.(Phe21Leu) | 2    | –   | –    | 0.935     | 1    | 1              | C0        |
| 2          | c.985G>T | p.(Asp329Tyr) | 9    | 0.04| 1.000| 1.000     | 0.04 | 0              | C35       |
| 3          | c.776C>T | p.(Ser259Leu) | 8    | –   | –    | 0.928     | 0.04 | 1              | C15       |
| 4          | c.769A>T | p.(Ile257Phe) | 8    | 8   | 8    | 1.000     | 0.04 | 1              | C15       |
| 5          | c.776C>T | p.(Ser259Leu) | 8    | –   | –    | 0.928     | 0.04 | 1              | C15       |
| 6          | c.985G>T | p.(Asp329Tyr) | –    | –   | –    | –         | –    | –              | –         |
| 7          | –    | –       | –    | –   | –    | –         | –    | –              | –         |

ExAC exome aggregation consortium, MAF minor allele frequency, PolyPhen2 polymorphism phenotyping v2, SIFT sorting intolerant from tolerant, “–” indicates that no data are available.
variants described remains relatively small to draw conclusions.

Based on the current classification of lissencephalies, the majority of the patients with TUBG1 variants fit within subtype 2–3. The predicted clinical outcome based on the classification is concordant with the phenotype in our patients [11].

It is not yet possible to identify a particular causative tubulin gene or variant based on clinical and radiologic presentations alone, as findings are not specific enough.

Figure 3: Distribution of the variants in the TUBG1 gene. Linear (a) and 3D (b) representation of the TUBG1 gene showing its functional domains and the distribution of the described TUBG1 variants. The variant in red in a represents the recurrent variant detected in four patients. The 3D structure is based on PDB ID: 3CB2 (crystal structure of gamma-tubulin bound to GDP) using NGL viewer [38, 39].
However, variants in TUBG1 should be considered as a possible differential diagnosis in patients presenting with posterior predominant pachygyria with no or minimal involvement of other brain structures, especially if variants in LIS1 have been ruled out.

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Author contributions

WBD, NDD, KS, and AJ conceived the work. SB, ES, MD, KK, and RG assisted with data acquisition. SB, KS, NDD, RG, and AJ drafted the manuscript, which was revised and approved by all.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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