doi:10.1093/brain/aws345 Brain 2013: 136; 522–535 | 522

BRAIN
A JOURNAL OF NEUROLOGY

A novel syndrome caused by the E410K amino acid substitution in the neuronal β-tubulin isotype 3

Sheena Chew,[1,2,3,4,5] Ravikumar Balasubramanian,[6,7] Wai-Man Chan,[1,2,5,8] Peter B. Kang,[1,3] Caroline Andrews,[1,2,3,5,8] Bryn D. Webb,[9,10] Sarah E. MacKinnon,[11] Darren T. Oystreck,[11,*,#] Jessica Rankin,[1,4,§] Thomas O. Crawford,[12] Michael Geraghty,[13] Scott L. Pomeroy,[1,2,3,14] William F. Crowley Jr,[6,7] Ethylin Wang Jabs,[9,10,15] David G. Hunter,[11] Patricia E. Grant[16] and Elizabeth C. Engle[1,2,3,5,8,11,14,17,18]

1 Department of Neurology, Boston Children's Hospital, Boston, MA 02115, USA
2 FM Kirby Neurobiology Centre, Boston Children's Hospital, Boston, MA 02115, USA
3 Department of Neurology, Harvard Medical School, Boston, MA 02115, USA
4 Harvard Medical School, Boston, MA 02115, USA
5 Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
6 Harvard Reproductive Endocrine Sciences Centre, Massachusetts General Hospital, Boston, MA 02114, USA
7 The Reproductive Endocrine Unit of the Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, USA
8 Program in Genomics, Boston Children's Hospital, Boston, MA 02115, USA
9 Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY, 10029, USA
10 Department of Pediatrics, Mount Sinai School of Medicine, New York, NY, 10029, USA
11 Department of Ophthalmology, Boston Children's Hospital, Boston, MA 02115, USA
12 Department of Neurology, Johns Hopkins Hospital, Baltimore, MA 21287, USA
13 Department of Paediatrics, Children's Hospital Eastern Ontario, University of Ottawa, Canada
14 Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA
15 Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY, 10029, USA
16 Department of Radiology, Boston Children's Hospital, Boston, MA 02115, USA
17 The Manton Centre for Orphan Disease Research, Boston Children's Hospital, Boston, MA 02115, USA
18 Department of Medicine (Genetics), Boston Children's Hospital, Boston, MA 02115, USA

*Present address: Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia
#Present address: Division of Ophthalmology, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa
§Present address: Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA 02114, USA

Correspondence to: Elizabeth C. Engle, MD,
CLS14074,
Boston Children's Hospital,
300 Longwood Ave,
Boston,
MA 02115,
USA
E-mail: Elizabeth.engle@childrens.harvard.edu

Missense mutations in *TUBB3*, the gene that encodes the neuronal-specific protein β-tubulin isotype 3, can cause isolated or syndromic congenital fibrosis of the extraocular muscles, a form of complex congenital strabismus characterized by cranial nerve misguidance. One of the eight *TUBB3* mutations reported to cause congenital fibrosis of the extraocular muscles, c.1228G>A results in a TUBB3 E410K amino acid substitution that directly alters a kinesin motor protein binding site. We report the detailed phenotypes of eight unrelated individuals who harbour this *de novo* mutation, and thus define the 'TUBB3 E410K syndrome'. Individuals harbouring this mutation were previously reported to have congenital fibrosis of the extraocular muscles,

Received September 26, 2012. Revised October 29, 2012. Accepted November 9, 2012

facial weakness, developmental delay and possible peripheral neuropathy. We now confirm by electrophysiology that a progressive sensorimotor polyneuropathy does indeed segregate with the mutation, and expand the TUBB3 E410K phenotype to include Kallmann syndrome (hypogonadotropic hypogonadism and anosmia), stereotyped midface hypoplasia, intellectual disabilities and, in some cases, vocal cord paralysis, tracheomalacia and cyclic vomiting. Neuroimaging reveals a thin corpus callosum and anterior commissure, and hypoplastic to absent olfactory sulci, olfactory bulbs and oculomotor and facial nerves, which support underlying abnormalities in axon guidance and maintenance. Thus, the E410K substitution defines a new genetic aetiology for Moebius syndrome, Kallmann syndrome and cyclic vomiting. Moreover, the c.1228G>A mutation was absent in DNA from ~600 individuals who had either Kallmann syndrome or isolated or syndromic ocular and/or facial dysmotility disorders, but who did not have the combined features of the TUBB3 E410K syndrome, highlighting the specificity of this phenotype–genotype correlation. The definition of the TUBB3 E410K syndrome will allow clinicians to identify affected individuals and predict the mutation based on clinical features alone.

Keywords: Kallmann syndrome; cyclic vomiting; peripheral neuropathy; CFEOM; TUBB3

Abbreviation: CFEOM = congenital fibrosis of the extraocular muscles

Introduction

Microtubules serve essential cellular functions critical for the development and maintenance of the mammalian nervous system. They provide structure and generate forces needed for neurons to migrate and to develop axonal and dendritic processes, and they provide organized scaffolds on which kinesin and dynein molecular motor proteins transport cargo. In recent years, it has been found that genetic mutations in their building blocks, the α- and β-tubulin isotypes, alter microtubule function in humans and cause neurological disorders (Keays *et al.*, 2007; Abdollahi *et al.*, 2009; Jaglin *et al.*, 2009; Kumar *et al.*, 2010; Poirier *et al.*, 2010; Tischfield *et al.*, 2010).

We previously reported that eight hot-spot missense mutations in *TUBB3*, which alter six amino acid residues in the neuronal β-tubulin isotype 3 (TUBB3), cause congenital fibrosis of the extraocular muscles (CFEOM), a complex congenital ocular dysmotility disorder characterized by ptosis and strabismus, with or without additional neurological findings (Tischfield *et al.*, 2010). We provided a brief overview of the clinical manifestations among 29 mutation-positive families and noted apparent correlations between specific missense mutations and the resulting phenotypes. We also argued that the phenotypes were at least in part caused by a dominant effect of mutant TUBB3 protein on axon guidance and maintenance and showed that the TUBB3 R262C substitution, which causes isolated CFEOM in humans, causes errors in axon guidance in a mouse model of the disease. Furthermore, we showed that all eight mutations altered microtubule dynamics *in vitro*, while a subset also perturbed binding of kinesin to the microtubule.

Within our initial description of the CFEOM-associated TUBB3 syndromes, we briefly summarized the phenotype of six individuals harbouring the *de novo* c.1228G>A *TUBB3* mutation, which directly alters a kinesin-binding site residue located within the H12 helix of TUBB3 and results in a TUBB3 E410K amino acid substitution. We reported that these individuals had severe CFEOM, facial weakness and developmental delay with variable thinning of the corpus callosum and anterior commissure, while the oldest may have been developing a sensorimotor polyneuropathy. We have now performed detailed phenotyping of these six patients and two additional previously unreported individuals, each harbouring a *de novo TUBB3* c.1228G>A missense mutation. We report that, in addition to the findings aforementioned, this mutation causes Kallmann syndrome, cyclic vomiting, vocal cord paralysis and dysmorphic facial features, including midface hypoplasia. This constellation of findings defines a distinctive 'TUBB3 E410K syndrome' that can be recognized at the bedside, leading to the rapid genetic diagnosis of affected individuals.

Materials and methods

Approximately 400 mutation-negative probands with isolated or syndromic congenital complex ocular dysmotility and/or congenital facial weakness and ~200 probands with isolated or syndromic Kallmann syndrome, were screened for the *TUBB3* c.1228G>A missense mutation (p.E410K). Eight probands were identified and are included in this report. After informed consent, participants enrolled in an ongoing research study at Boston Children's Hospital, and a subset also enrolled in ongoing research studies at Massachusetts General Hospital and Mount Sinai School of Medicine, with approval by the corresponding institutional review boards. Mutation analysis of six subjects was previously reported (Tischfield *et al.*, 2010). Two previously unreported subjects provided a blood sample from which genomic DNA was extracted using Gentra® Puregene® Blood Kits (Qiagen), and the *TUBB3* gene was sequenced as previously described (Tischfield *et al.*, 2010). Maternity and paternity were confirmed by the appropriate inheritance of at least 10 informative polymorphic markers.

Detailed medical and family histories and clinical and neuroimaging data were obtained from participants, medical records and dedicated research visits. Olfaction was evaluated by administration of either the Smell Identification Test (Philadelphia, PA) or the Alcohol Sniff Test (San Diego, CA) when feasible, or documented by history. Facial 3D images for morphometric analysis were attained using a 3DMD system (http://3dmd.com), and measurements were made using a freehand point selection option and repeated to ensure reliability. Coding exons and intron–exon boundaries of previously known Kallmann syndrome genes *CHD7*, *KAL1*, *PROK2*, *PROKR2*, *NELF*, *FGF8* and *FGFR1* were amplified from genomic DNA from five of the eight subjects using published primer sequences and PCR conditions (Jongmans *et al.*,

2009; Sykiotis *et al.*, 2010), and PCR products were sequenced and analysed.

Results

Four male and four female individuals of diverse ethnic backgrounds ranging from 7 to 25 years of age were found to harbour the *TUBB3* c.1228G>A mutation (p.E410K). In each, the heterozygous mutation arose *de novo*. Two pedigrees (ZY and AER) are previously unpublished (Fig. 1), whereas six were presented previously (Tischfield *et al.*, 2010). At the time of enrolment, seven had been diagnosed with Moebius syndrome and one with bilateral oculomotor and facial nerve palsies. Subjects are indicated by roman numerals in the order of chronological age from youngest to oldest, and each subject's clinical findings are highlighted later in the text and summarized in Table 1.

Perinatal findings

All eight subjects had ptosis, exotropia and facial weakness at birth, and diagnosed within the first 5 months of life. Three male subjects were diagnosed with cryptorchidism and/or microphallus during infancy. Four subjects had stridor as infants, of whom three underwent direct lanyngoscopy for tracheomalacia or laryngomalacia, two were found to have bilateral vocal cord paralysis and one required tracheostomy for the first 7 years of life.

Growth and development

Seven subjects had short stature (3rd–25th percentile) until adolescence, after which the older male subjects failed to undergo the pubertal growth spurt and dropped below the third percentile. Three subjects had microcephaly. Growth charts are displayed in Fig. 2. All subjects have required special education for delayed intellectual and/or social development, and those tested have full-scale IQ scores between 50 and 90. Subject II developed complex partial seizures at age 3 years, and EEG revealed left centrotemporal and right frontal sleep-activated spikes. Five subjects had fine and gross motor delays. Six had hypotonia and one had hypertonia early in life. Five developed lower-extremity clonus.

Dysmorphology and general examinations

Five subjects underwent 3D photography and were found to have midface flattening, a non-prominent and short nose, a short smooth philtrum and low set and/or posteriorly rotated ears (Table 2, representative images shown in Fig. 3). Subjects IV and VII had lumbar and thoracic scoliosis. None had cleft palate, renal agenesis, vertebrae or limb defects or mirror movements. Baseline cardiac, respiratory and abdominal examination results were unremarkable.

Cranial nerve abnormalities

Subjects demonstrated clinical abnormalities of the olfactory, oculomotor, facial and vagal nerves as summarized in Table 1.

Olfactory nerve

The seven subjects tested had anosmia, and neuroimaging of all eight subjects revealed olfactory sulcus and olfactory bulb dysgenesis (Fig. 4).

Oculomotor, trochlear and abducens nerves

All subjects had a severe form of CFEOM, with congenital bilateral ptosis, globe infraduction and exotropia ranging from 20 to 100 prism dioptres and minimal to no eye movements (Fig. 3). Residual eye movements in four subjects were aberrant (Table 3). Oculomotor nerves were hypoplastic or not as visualized by neuroimaging (Fig. 5), similar to individuals harbouring TUBB3 R262C or D417N substitutions who have oculomotor and optic nerve hypoplasia, and variable extraocular muscle atrophy (Demer *et al.*, 2010). The severe exotropia and hypotropia of the subjects' eyes limited the ability to assess trochlear and abducens function,

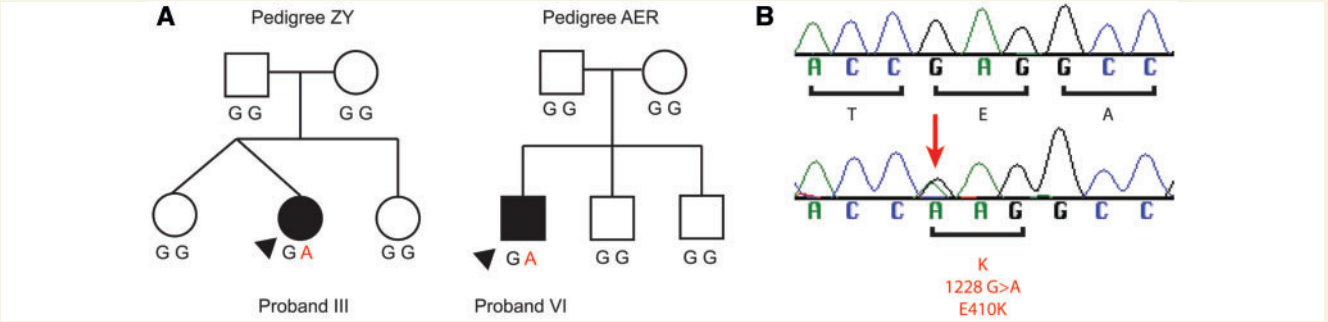

Figure 1 The TUBB3 c.1228G>A mutation (p.E410K) arose *de novo* in Pedigrees ZY and AER. (**A**) Schematic structures of Pedigrees ZY and AER. Squares denote male subjects, circles denote female subjects and filled symbols indicated by the black triangles denote Probands III and VI. The absence or presence of the c.1228G>A mutation is indicated by a G or A, respectively. (**B**) Chromatograms from a control individual (*top*) and a proband showing the c.1228G>A heterozygous mutation (*bottom*). The nucleotide substitution is represented by the double peak and indicated by a red arrow. The corresponding normal and mutated amino acid residues are indicated with brackets under each codon triplet.

Table 1 Clinical characteristics of patients with TUBB3 E410K substitution

Subject	I	II	III	IV	V	VI	VII	VIII
Pedigree	0007	LC	ZY	RZ	CP	AER	MJ	AGE
Age (years) and gender (M/F)	7 F	9 M	9 F	15 F	19 F	20 M	21 M	25 M
Ethnicity/ancestry	CME	CME	Caucasian, Ashkenazi Jewish	African, Senegalese	Caucasian, Turkish	CME	Asian/CME	CME
Development								
IUGR	–	–	+	–	–	–	+	–
Foetal distress	–	–	+	–	–	+	+	–
Stridor			+			+	+	+
Tracheomalacia	–	–	+	–	–	+	+	
Tracheostomy	–	–	–	–	–	–	+	–
Facial dysmorphism		+	+	+		+	+	+
Microcephaly	–	+	+	–	–	–	+	–
Intellectual disability	~/+	+	+	+	+	+	+	+
Seizures	–	+	–	–	–	–	–	–
Corpus callosum abnormalities	+ +	+	+ +	+	+ + +	+ +	+ +	+ +
Cranial nerves								
Cranial nerve I	+	+	+	+		+	+	+
Anosmia/hyposmia								
Cranial nerve III	+	+	+	+	+	+	+	+
Ptosis, exotropia, restricted eye movements								
Cranial nerve VII	+	+	+	+	+	+	+	+
Bilateral facial weakness								
Cranial nerve X	–	–	–	–	–	+	+	–
Vocal cord paralysis								
Endocrine								
Hypogonadotropic hypogonadism		+		+	+	+	+	+
Olfactory sulcus dysgenesis	+	+	+	+	+	+	+	+
Olfactory bulb agenesis	+ +	+ +	+	+ +	+ +	+	+ +	+ +
Microphallus		+				–	+	–
Cryptorchidism		+				–	+	+
Gastrointestinal								
Recurrent vomiting	–	+	–	–	–	+ +	+ + +	+ + +
Onset (years of age)	–	8	–	–	–	<10	4	20
Neuromuscular								
Clinical symptoms of peripheral neuropathy	–	–	–	–	–	–	+	+
EMG/NCS abnormal		+	–	+	+		+	

CME = Caucasian of mixed European descent; M = male; F = female; IUGR = intrauterine growth retardation; NCS = nerve conduction studies.

and imaging data did not permit definitive evaluation of the presence or absence of these nerves.

Facial nerve

All eight subjects had bilateral facial paralysis including the forehead, and facial nerves were not visible by neuroimaging (Fig. 5). Salivation was intact in all subjects, and gustatory sensation was normal in the two subjects tested.

Vagus nerve

Two subjects were diagnosed with vocal cord paralysis as infants.

Remaining cranial nerves

No subject was noted to have optic nerve hypoplasia or retinal anomalies; none, however, had visual acuity of 20/20 or better, and all but one tested subject had visual acuity of 20/40 or worse in the better-seeing eye (Table 3). Slit-lamp examination results were normal, with the exception of corneal exposure in five subjects. All had normal facial sensation, hearing, chewing and swallowing, as well as normal midline palate elevation, strong sternocleidomastoid muscles and symmetric tongue movement with no signs of tongue atrophy or fasciculation.

Peripheral neuropathy

The two oldest subjects reported sensory and/or motor changes in their distal lower extremities. Nerve conduction studies and/or electromyography studies were performed on five subjects between the ages of 9 and 21 years. Three had mild sensory and motor nerve conduction study abnormalities, a fourth had primarily sensory abnormalities and the youngest had essentially normal nerve conduction studies (Table 4). The abnormal conduction velocities were generally ~80% of the lower limit of normal, which is the threshold distinguishing demyelinating from axonal polyneuropathies.

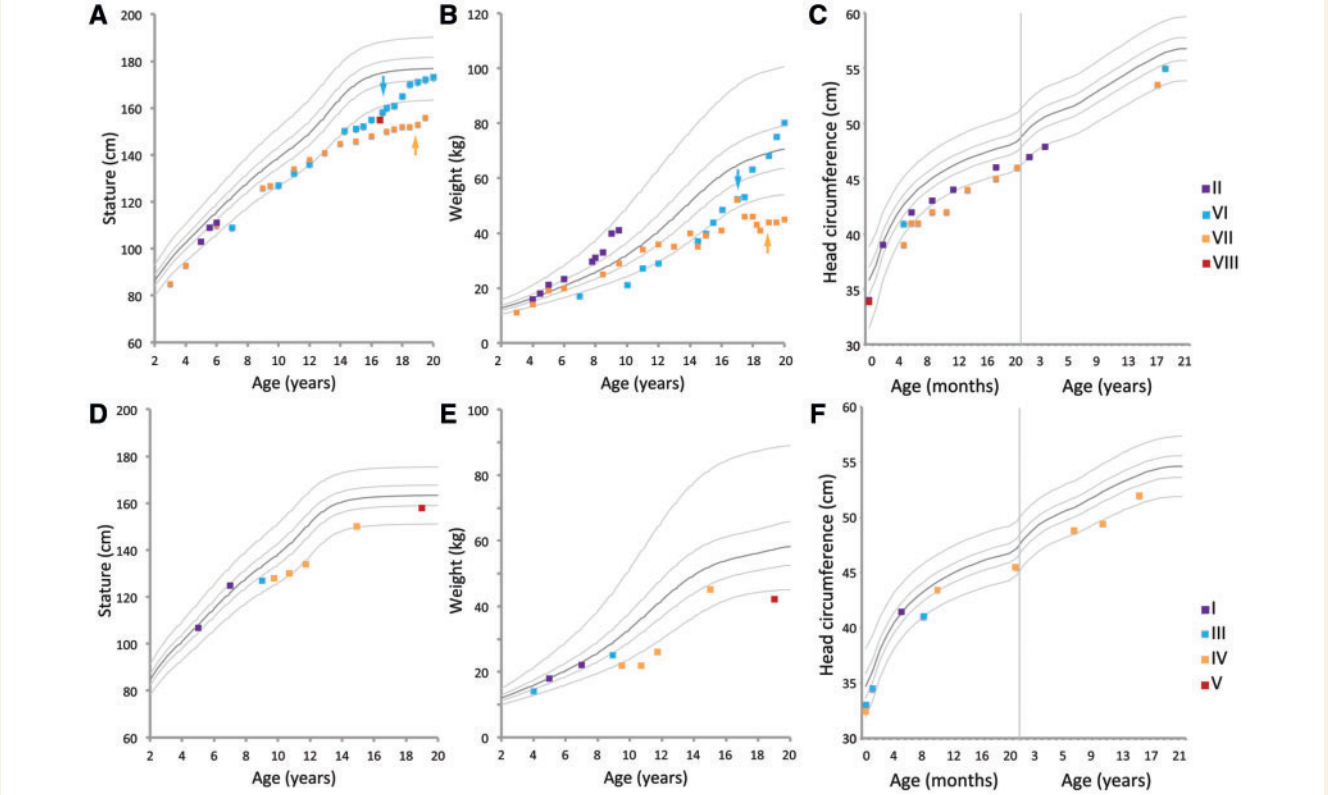

Figure 2 Growth parameters for individuals harbouring the TUBB3 E410K substitution. Stature (**A** and **D**), weight (**B** and **E**) and head circumference (**C** and **F**) for age for the male subjects (*top row*) and female subjects (*bottom row*). The grey standard curves represent the 3rd, 25th, 50th, 75th and 97th percentiles. Stature and weight standard curves created with percentile data from Centers for Disease Control National Health Statistics, available at http://www.cdc.gov/growthcharts/clinical_charts.htm#Set2. Head circumference standard curves created with data from (Rollins *et al.*, 2010). Blue and orange arrows indicate the start of testosterone therapy for hypogonadotropic hypogonadism for Subjects VI and VII, respectively.

Hypogonadotropic hypogonadism

All four male subjects and the two oldest female subjects were diagnosed with Kallmann syndrome, a distinctive phenotypic form of idiopathic hypogonadotropic hypogonadism that is defined by the combined occurrence of gonadotropin-releasing hormone deficiency and defective olfactory function (Bianco and Kaiser, 2009; Crowley, 2011). Two female subjects were too young to be evaluated for hypogonadotropic hypogonadism, but both had anosmia. The subjects diagnosed with Kallmann syndrome had absent or delayed puberty, low gonadotropin and sex steroid levels and otherwise normal pituitary function (Table 5). Three male subjects also had micropenis and/or cryptorchidism, consistent with neonatal gonadotropin-releasing hormone deficiency. Hypogonadotropic hypogonadism is genetically heterogeneous and can be oligogenic (Balasubramanian *et al.*, 2010; Tornberg *et al.*, 2011). To exclude oligogenic inheritance, we sequenced the known Kallmann syndrome genes in the five subjects for whom we had sufficient DNA. Polymorphisms, but no other putative disease-causing variants, were identified (Table 6).

Cyclic vomiting syndrome

The two oldest male subjects (VII and VIII), were diagnosed with cyclic vomiting syndrome based on recurrent attacks of severe nausea and vomiting beginning at ages 4 and 20 years, respectively, that had no clear causal aetiology and were separated by periods of baseline health (Li *et al.*, 2008). Each had multi-day episodes of severe vomiting triggered by foods, infections or strong emotions. Episodes would be heralded by a prodrome of several hours, after which vomiting would occur every few minutes, accompanied by nausea, photophobia, phonophobia and incontinence, but no headache. Subject VII also had signs suggestive of dysautonomia, including sinus arrhythmia during vomiting episodes, difficulty with body temperature regulation and excessive sweating. EEG monitoring, gastrointestinal anatomical and gastric emptying study results were normal during and between events.

For both subjects, prophylactic valproic acid at a therapeutic serum level of 50–100 mcg/ml reduced the frequency and severity of episodes. Episodes could often be aborted by aprepitant during the prodromal phase. A combination of intranasal sumatriptan, promethazine suppository and rectal diazepam controlled symptoms if administered immediately after onset of vomiting; otherwise, intravenous chlorpromazine, fluids and sedation were required.

The two other male subjects had features of cyclic vomiting syndrome. Subject VI had vomiting episodes several times a year

Table 2 Facial morphometric analysis of individuals with TUBB3 E410K substitution

Subject	II	III	IV	VI	VII
Age at evaluation	9 years 6 months	8 years 3.6 months	15 years 4 months	19 years 2.6 months	21 years 3 months
Head size	Microcephalic (<5%)	Microcephalic (<5%)	Normocephalic (10%)	Normocephalic (10–25%)	Microcephalic (<5%)
Palpebral fissures	Upslanting, epicanthal folds	Unremarkable	Upslanting, epicanthal folds	Unremarkable	Epicanthal folds
Ears	Unremarkable	Slightly low set	Right ear posteriorly rotated	Slightly low set, posteriorly rotated	Unremarkable
Facial asymmetry	Unremarkable	Unremarkable	Unremarkable	Asymmetric	Unremarkable
Midface	Midface flattening	Midface flattening	Unremarkable	Midface flattening	Unremarkable
Nose	Non-prominent, upturned, short	Non-prominent, short, non-protruding	Broad, flat nasal bridge, short, non-protruding	Non-prominent, short	Non-prominent
Nasal length	4.18 cm (−2 to −1 SD)	3.5 cm (−2 SD)	4.19 cm (> −2 SD)	4.6 cm (> −2 SD)	5.25 cm (mean)
Nasal protrusion	1.6 cm (mean)	1.2 cm (> −2 SD)	1.56 cm (> −2 SD)	1.9 cm (−1 SD)	1.81 cm (−2 to −1 SD)
Nasal width	2.97 cm (−1 SD to mean)	2.88 cm (−1 SD to mean)	3.89 cm (> +2 SD)	3.37 cm (mean for 16 yo)	3.19 cm (−1 SD for 16 yo)
Philtrum	Smooth	Short, smooth	Short, smooth	Smooth	Smooth
Philtrum length	1.23 cm (25th percentile)	1.1 cm (> −2 SD)	0.71 cm (> −2 SD)	1.5 cm (−1–2 SD for 16 yo)	1.46 cm (−1–2 SD for 16 yo)
Intercommisural distance	4.23 cm (−1 SD to mean)	4.1 cm (−1 SD)	4.9 cm (mean)	5.0 cm (mean for 16 yo)	4.69 cm (−1–2 SD for 16 yo)

Normal values from Hall *et al.* (1989), Saul *et al.* (1998) and Zankl *et al.* (2002). Values in parentheses refer to percentiles for age.
SD = standard deviation; yo = years old.

triggered by illness and lasting for several days. Subject II had sporadic vomiting episodes triggered by anxiety and characterized by early-morning awakening with abdominal discomfort, conjunctival injection and nausea, followed by a brief period of unresponsiveness and limpness with subsequent vomiting.

Discussion

We have determined that the *TUBB3* c.1228G>A missense mutation, which typically arises *de novo*, causes a constellation of congenital and degenerative phenotypes that we have named the 'TUBB3 E410K syndrome'. The TUBB3 E410K syndrome consists of intellectual disability, Kallmann syndrome, CFEOM, facial weakness, tracheomalacia, vocal cord paralysis and later-onset cyclic vomiting and progressive peripheral neuropathy. Neuroimaging reveals thin corpus callosa and anterior commissures, as well as hypoplastic to absent olfactory sulci, olfactory bulbs and oculomotor and facial nerves, which are consistent with abnormalities in axon guidance and maintenance. These findings expand significantly our initial report of the clinical manifestations resulting from this mutation and permit its recognition as a human malformation syndrome distinct from the phenotypes resulting from other reported TUBB3 amino acid substitutions, including D417H, D417N and R262C that, like E410K, also alter kinesin–microtubule interactions (Tischfield *et al.*, 2010). These findings also highlight the specificity of the TUBB3 E410K genotype–phenotype correlation; none of the ~600 *TUBB3* mutation-negative individuals who had either Kallmann syndrome or ocular and/or facial dysmotility disorders had the combined features of the TUBB3 E410K syndrome.

Individuals harbouring the TUBB3 E410K syndrome may be diagnosed with Moebius syndrome

Moebius syndrome (Moebius, 1888) is defined by congenital limitations of eye abduction and facial movement. Affected individuals classically have eyes that are straight or esotropic in the primary position, full vertical eye movements and limitations in abduction. Seven of the subjects in our study cohort carried the diagnosis of Moebius syndrome at the time of enrollment, but had atypical features because their eyes were exotropic and infraducted bilaterally with minimal to absent vertical eye movements and severe limitations in adduction that exceeded limitations in abduction.

A series of case reports have described genetically undefined individuals diagnosed with Moebius syndrome who, like our cohort of eight subjects, have ptosis, horizontal and vertical gaze limitations and facial weakness accompanied by hypogonadotropic hypogonadism and peripheral neuropathy (Olson *et al.*, 1970; Rubinstein *et al.*, 1975; Abid *et al.*, 1978; Kawai *et al.*, 1990; Brackett *et al.*, 1991; Jennings *et al.*, 2003), intellectual disability (Olson *et al.*, 1970; Abid *et al.*, 1978; Brackett *et al.*, 1991), thin corpus callosum (Brackett *et al.*, 1991) and/or facial dysmorphism with small nose (Rubinstein *et al.*, 1975; Brackett *et al.*, 1991). Thus, these individuals likely had the TUBB3 E410K syndrome. We

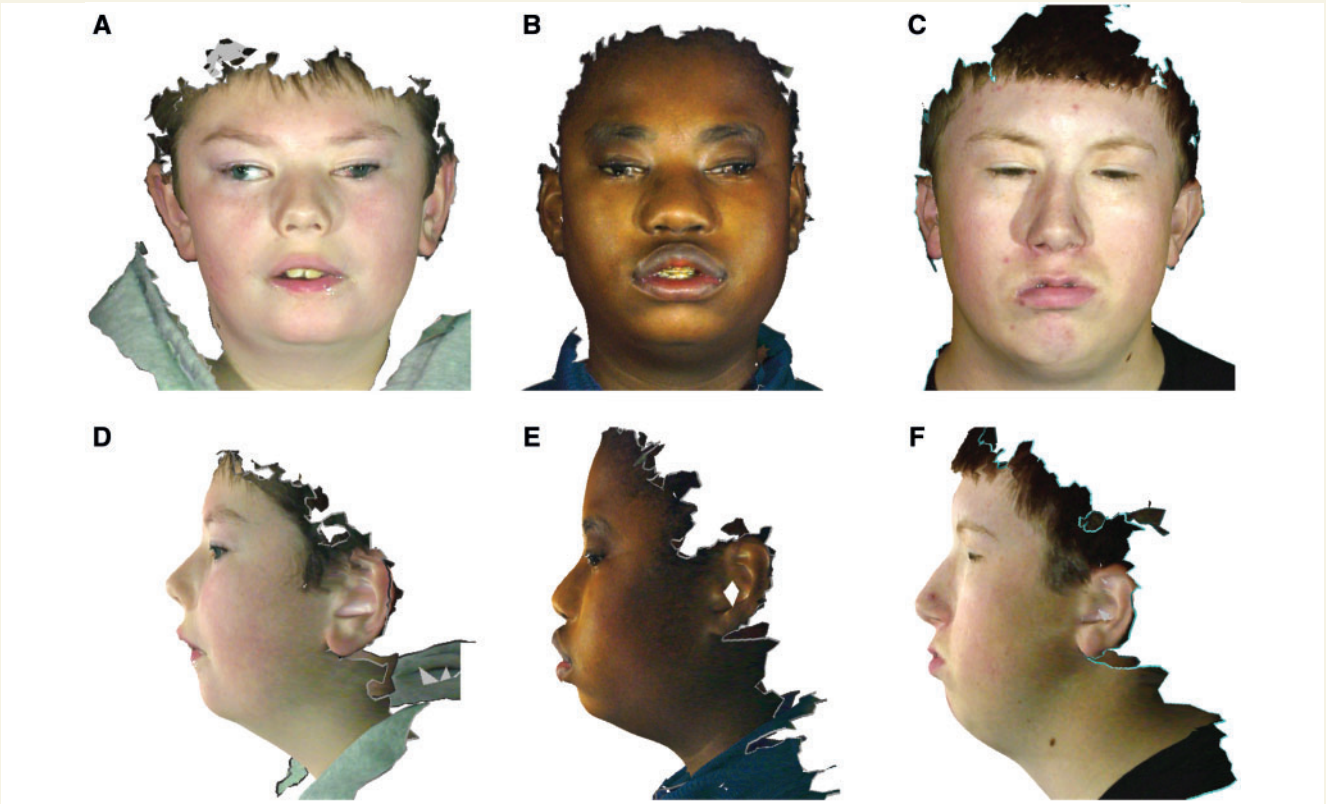

Figure 3 3D morphometric analysis of individuals with the TUBB3 E410K substitution. 3D photographs were obtained for five subjects, and frontal (**A–C**) and profile (**D–F**) views of three subjects are shown. Generally, patients with the TUBB3 E410K substitution are noted to have ptosis, globe infraduction and exotropia, a short nose, a short smooth philtrum, midface flattening and a mask-like face. (**A** and **D**) Subject is noted to have ptosis, upslanting palpebral fissures and epicanthal folds. He has a short upturned nose and midface flattening. His philtrum is smooth. (**B** and **E**) Subject is noted to have ptosis, upslanting palpebral fissures and epicanthal folds. Her nose is short, non-protruding and broad. She has a flat nasal bridge and short smooth philtrum. (**C** and **F**) Subject is noted to have ptosis and facial asymmetry. He has a short nose and midface flattening. His philtrum is smooth, and his ears are slightly low set and posteriorly rotated.

anticipate that our definition of this syndrome will lead to its increased recognition and subsequent testing for the *TUBB3* c.1228G>A mutation among Moebius patient populations worldwide.

The TUBB3 E410K substitution causes maldevelopment of a subset of cranial nerves

Clinically, the TUBB3 E410K substitution affects specific cranial nerves and, in some instances, selective nerve branches. Hypoplastic oculomotor and facial nerves are consistent with congenital cranial nerve maldevelopment, and clinical signs of aberrant extraocular muscle innervation support axon misguidance as the pathological basis for at least some of the TUBB3 E410K phenotypes. We note that some individuals also had vocal cord paralysis, consistent with dysfunction of the recurrent laryngeal branch of the vagal nerve. Preserved control of pupillary constriction, salivation and gustatory sensation suggests that the somatic/branchial motor efferent cranial nerve fibres are more vulnerable than visceral efferent or special sensory afferent fibres. Although facial sensation, taste and hearing appear to be intact, the TUBB3 E410K substitution does not spare all sensory nerves, as dysfunction of the olfactory special sensory afferents results in anosmia.

The TUBB3 E410K substitution defines a new genetic aetiology of Kallmann syndrome

We have identified that the TUBB3 E410K substitution is a novel genetic aetiology of Kallmann syndrome (Kallmann *et al.*, 1944), as the subjects had idiopathic hypogonadotropic hypogonadism and clinical and/or radiological evidence of abnormal olfaction. Hypogonadotropic hypogonadism results from a failure of pulsatile secretion of gonadotropin-releasing hormone required for humans to initiate puberty (Balasubramanian *et al.*, 2010). Male subjects with the E410K substitution had microphallus/cryptorchidism as neonates, while subjects of both sexes had a gonadotropin-releasing hormone deficiency phenotype at the time of puberty. Subject VII displayed a form of partial pubertal development known as the 'fertile eunuch' variant of idiopathic hypogonadotropic hypogonadism, characterized by spontaneous testicular maturation in the face of overt hypogonadism and implying a degree of testicular development (Pitteloud *et al.*, 2001, 2002).

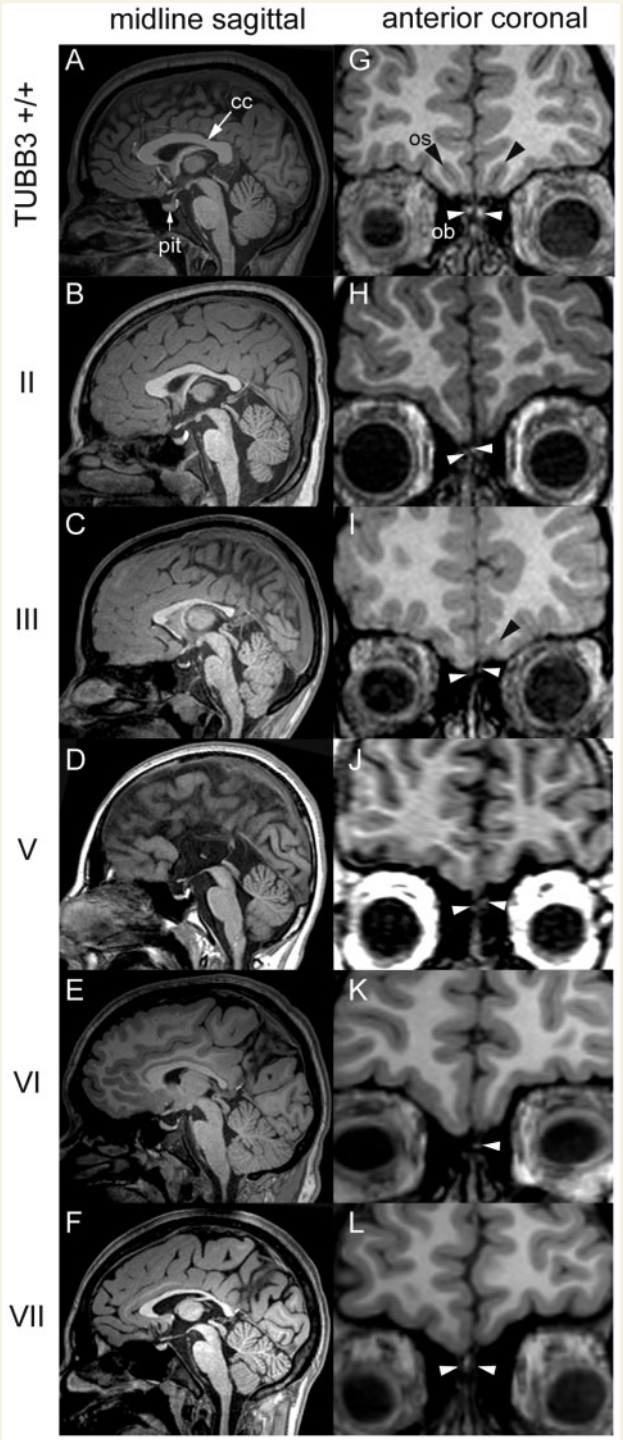

Figure 4 Midline sagittally acquired (**A–F**) and coronal reformations (**G–L**) of volumetric T_1-weighted magnetic resonance images demonstrate abnormalities of the corpus callosum, pituitary gland, olfactory sulci and olfactory bulbs in individuals with the TUBB3 E410K substitution. (**A**) Corpus callosum (cc) and pituitary gland (pit) in a control individual. (**B–F**) The TUBB3 E410K substitution produces variable corpus callosum abnormalities, including diffuse thinning (**C** and **F**), hypoplasia of the posterior body (**B**), rostral dysgenesis with posterior hypoplasia (**E**) and complete agenesis (**D**). The pituitary gland appears small in all subjects, as can be seen in Kallmann syndrome. (**G**) Olfactory sulci (black arrowhead, os) and olfactory bulbs (white arrowheads, ob) in a control individual. (**H–L**) Olfactory sulcus agenesis is seen in all individuals with the TUBB3 E410K substitution except Subject III, who has unilateral olfactory sulcus dysgenesis (single abnormal olfactory sulcus is indicated by the black arrowhead) (**I**). Most individuals with the TUBB3 E410K substitution demonstrate bilateral olfactory bulb dysgenesis (highlighted by white arrowheads) (**H–L**). Subject VI (**K**) has left olfactory bulb hypoplasia (white arrowhead). The right olfactory bulb is not visible by neuroimaging. Voxel size, 1.1458 × 1.1458 × 1.0000 mm.

Thus, the TUBB3 E410K subjects display some phenotypic heterogeneity at the time of puberty, as is typical of most genetic causes of idiopathic hypogonadotropic hypogonadism (Balasubramanian *et al.*, 2010). Although synergistic interactions between multiple mutated disease genes are thought to contribute to this phenotypic heterogeneity, we did not find mutations in other genes, and the TUBB3 E410K substitution alone appears sufficient.

The TUBB3 E410K substitution likely causes Kallmann syndrome by disrupting olfactory axon guidance and gonadotropin-releasing hormone cell migration from the nasal placode to the olfactory bulb. This would be consistent with the proposed aetiology for other genetic forms of Kallmann syndrome (Balasubramanian and Crowley, 2011) and with the deleterious effect of TUBB3 E410K mutant protein on the development of other cranial nerves in humans, and on the guidance of oculomotor axons that we reported in TUBB3 R262C homozygous mice (Tischfield *et al.*, 2010).

The TUBB3 E410K substitution implicates TUBB3 as the first cytoskeletal protein mutated in Kallmann syndrome and suggests a common final pathway for Kallmann syndrome pathogenesis that converges on the microtubule. Notably, one of six *TUBB3* mutations reported to cause polymicrogyria in the absence of CFEOM was harboured by a foetus, and pathological findings included olfactory bulb agenesis (Poirier *et al.*, 2010). Moreover, some *TUBA1A* mutations have been reported to cause olfactory sulcus abnormalities in the setting of lissencephaly (Fallet-Bianco *et al.*, 2008). Thus, mutations in other tubulin isotypes may also cause Kallmann syndrome.

The TUBB3 E410K substitution defines a genetic aetiology of cyclic vomiting syndrome

Cyclic vomiting syndrome is a rare disorder, with a childhood prevalence of 0.4–1.9% (Li and Balint, 2000; Ertekin *et al.*, 2006). Its aetiology and pathophysiology are poorly understood. Mitochondrial DNA mutations altering cellular energy production have been proposed to cause both cyclic vomiting syndrome and migraine headaches (Boles *et al.*, 2003; Wang *et al.*, 2004), and one family with cyclic vomiting was reported to harbour a maternally inherited mutation in the mitochondrial transfer RNA gene *MT-TL1* (Salpietro *et al.*, 2003). Other proposed mechanisms include autonomic dysfunction (Chelimsky and Chelimsky, 2007) and dysregulation of the hypothalamic corticotropin-releasing hormone response to stress (Tache, 1999). Although cyclic vomiting

Table 3 Ophthalmological characteristics of subjects with TUBB3 E410K substitution

Subject	I	II	III	IV	V	VI	VII	VIII
Pupils	Reactive	Reactive	Small, reactive	Reactive	ND	Small, reactive	Sluggish, anisocoria	ND
Corrected visual acuity	20/60 OD 20/60 OS	20/50 OD 20/60 OS	ND	20/125 OD 20/160 OS	20/25 OD 20/30 OS	20/50 OD 20/40 OS	20/80 OD 20/70 OS	20/40 OD 20/200 OS
Ptosis	OU	OU mild	OU	OU mild	OU	OU	OU	OU
Ocular alignment	ID, large XT	ID, large XT	ID, moderate XT	ID, large XT	ID, large XT	ID, moderate XT	ID, large XT	ID, large XT
Adduction	Absent	Absent	Absent	Absent	Absent	Mild limitation	Absent	Absent
Vertical movements	Minimal	Minimal	Minimal	Absent	Minimal	Absent	Absent	Minimal
Aberrant movements	ND	Left jaw winking	Horizontal movements with attempted vertical gaze	ND	ND	Lid elevation with abduction	Synergistic divergence	ND
Corneal exposure	+	+				+	+	+

Eyes of all patients were fixed in infraduction.
Corneal exposure = history of problems with corneal exposure requiring treatment; ID = infraducted; ND = no data; OD = oculus dexter (right eye); OS = oculus sinister (left eye); OU = oculi uterque (both eyes); XT = exotropia.

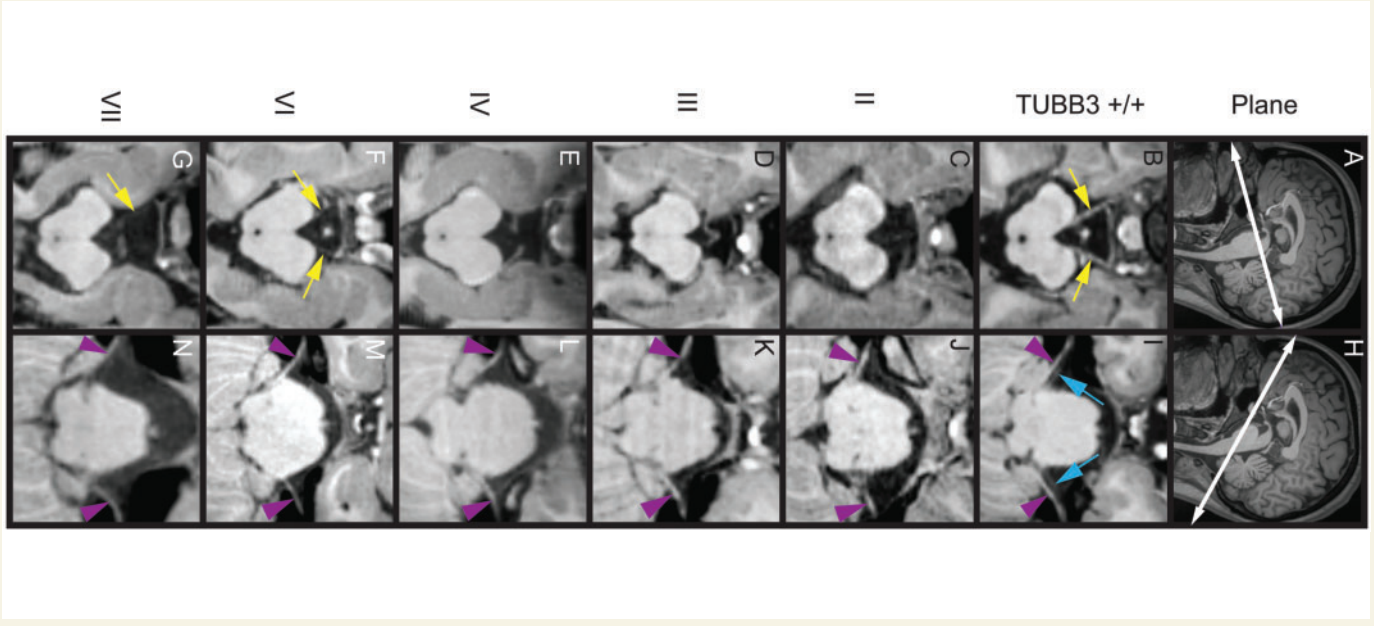

Figure 5 Oblique reformations of sagittally acquired volumetric T_1-weighted images oriented to the plane of selected cranial nerves demonstrate oculomotor and facial nerve hypoplasia in TUBB3 E410K subjects. **(A)** Plane of images displayed in **(B–G)**. **(B)** Oculomotor nerves in a control individual are indicated by yellow arrows (arrowhead perpendicular to and touching the nerve). **(C–G)** Hypoplastic oculomotor nerves are visualized bilaterally in Subject VI **(F)** and unilaterally in Subject VII **(G)**, but not in Subjects II, III and IV **(C–E)**. **(H)** Plane of images displayed in **I–N**. **(I)** Vestibulocochlear nerves (purple arrowheads) and facial nerve (blue arrows) in a control individual. **(J–N)** Vestibulocochlear nerves (purple arrowheads) are visualized bilaterally in all subjects. Facial nerves are not visible by neuroimaging in these individuals, indicating absence or severe hypoplasia.

Table 4 Nerve conduction studies of five individuals with the TUBB3 E410K substitution

	Sensory						Motor							
Subject	Nerve	Side	Amp (μV)		Velocity (m/s)		Nerve	Side	Dist (ms)		Amp (mV)		Velocity (m/s)	
II	Median	L	**19.8**	(>20)	**40**	(>50)	Median	L	4.15	(<4.3)	9.6/9.6	(>4)	**42.9**	(>49)
	Ulnar	L	19.5	(>17)	**45.7**	(>50)	Ulnar	L	2.35	(<3.3)	8.6/6.5	(>6)	**36.1**	(>49)
	Sural	L	**NR**	(>6)	**NA**	(>40)	Peroneal	L	4.75	(<6.5)	4.1/3.5	(>2)	**33.6**	(>44)
	Peroneal	L	**NR**	(>6)	**NA**	(>40)	Tibial	L	**6.8**	(<5.8)	2.0/0.9	(>4)	**32.7**	(>41)
III	Median	L	**13.7**	(>20)	50	(>50)	Median	L	3.05	(<4.0)	8.0/7.9	(>4)	56.1	(>49)
	Sural	L	12.1	(>6)	55.6	(>50)	Peroneal	L	3.25	(<6.5)	6.4/6.4	(>2)	48.6	(>44)
IV	Median	R	24.9	(>20)	**39**	(>50)	Median	R	4.1	(<4.3)	13.2/13.4	(>4)	**41**	(>49)
	Ulnar	R	32.7	(>17)	**41**	(>50)	Ulnar	R	3.2	(<3.3)	7.6/6.6/7.0	(>6)	**46**	(>49)
	Sural	R	8	(>6)	**28**	(>40)	Peroneal	R	6.5	(<6.5)	6.0/5.2/5.3	(>2)	**35**	(>44)
	Peroneal	R	**2**	(>6)	**32**	(>40)	Tibial	R	**8.2**	(<5.8)	5.6/4.2	(>4)	**28**	(>41)
V	Median	R	**9.6**	(>20)	55	(>50)	Median	R	3.5	(<4.0)	11.4	(>4)	50	(>49)
	Ulnar	R	**5.4**	(>17)	55	(>50)	Ulnar	R	2.1	(<3.3)	7	(>6)	53	(>49)
	Sural	R	**NR**	(>6)	**NA**	(>40)	Peroneal	R	4.2	(<6.5)	2.9	(>2)	40	(>44)
	Sural	L	**NR**	(>6)	**NA**	(>40)	Peroneal	L	4.3	(<6.5)	1.9	(>2)	41	(>44)
							Tibial	R	3.2	(<5.8)	5.3	(>4)	40	(>41)
							Tibial	L	3.7	(<5.8)	6.1	(>4)	40	(>41)
VII	Median	L	**13**	(>20)	**40.7**	(>50)	Median	L	3.6	(<4.0)	11.8/9.3	(>4)	**39**	(>49)
	Ulnar	L	**4.9**	(>17)	**38.3**	(>50)	Ulnar	L	2.7	(<3.3)	10.1/8.4	(>6)	**36.9**	(>49)
	Sural	L	**NR**	(>6)	**NA**	(>40)	Peroneal	L	**NR**	(<6.5)	**NR**	(>2)	**NR**	(>44)
	Peroneal	L	**NR**	(>6)	**NA**	(>40)	Peroneal (TA)	L	2.95	(<6.7)	**4.4/4.2**	(>5)	**29.7**	(>44)
							Tibial	L	4.45	(<5.8)	**5.3**	(>4)	**NA**	(>41)

Abnormal values are in bold. Where multiple amplitudes are provided in a single line, the amplitudes are listed in order from distal to proximal stimulation points. Peroneal motor nerve conduction studies were recorded from extensor digitorum brevis unless otherwise noted. Normal values are modified from Preston and Shapiro, 2005.
Amp = amplitude; Dist = distal latency; L = left; R = right; NR = no response; NA = not applicable; TA = tibialis anterior.

can occur in isolation or with additional neurological findings such as cognitive disorders, cranial nerve dysfunction and seizures (Boles *et al.*, 2006), to our knowledge, it has not been previously identified as a feature of a defined clinical syndrome.

The TUBB3 E410K substitution is the first genetic and non-mitochondrial aetiology for cyclic vomiting identified in multiple unrelated individuals. The TUBB3 E410K substitution disrupts microtubule function in both central and peripheral neurons, and thus it is unclear whether it causes cyclic vomiting by perturbing autonomic nerve function, affecting CNS circuitry or a combination of both. However, cyclic vomiting appears to be a highly specific manifestation of neuronal dysfunction associated with the TUBB3 E410K substitution. Subjects with other *TUBB3* mutations who have similar or more severe abnormalities do not report cyclic vomiting, and we are not aware of cyclic vomiting syndrome associated with mutations in any other neurodevelopmental disease genes.

The TUBB3 E410K substitution defines a genetic aetiology of peripheral polyneuropathy

We previously suggested that the TUBB3 E410K substitution might cause peripheral neuropathy based on the oldest subject's clinical symptoms (Tischfield *et al.*, 2010). We have now established that the TUBB3 E410K syndrome includes progressive polyneuropathy with onset of nerve conduction abnormalities as early as the first decade of life, and clinical signs and symptoms in the third decade. The subjects' nerve conduction and electromyography studies demonstrated that the TUBB3 E410K polyneuropathy affects sensory fibres. Although motor fibres are likely also involved based on clinical presentation, more definitive evidence from needle electromyography is needed.

The E410K substitution now becomes the fourth TUBB3 substitution, along with R262H, D417H and D417N, recognized to cause a polyneuropathy, with age of onset and severity varying by amino acid substitution (Tischfield *et al.*, 2010). While *TUBB3* is highly expressed in neurons in both the developing central and peripheral nervous systems (Katsetos *et al.*, 2003), high levels of expression are maintained only in peripheral neurons (Jiang and Oblinger, 1992). This may account, in part, for this degenerative phenotype. Moreover, these four TUBB3 substitutions share specific characteristics: each permits mutant TUBB3 protein incorporation into microtubules at wild-type or near wild-type levels in HeLa cells, directly or indirectly disrupts tubulin–motor protein binding sites in the tubulin H12 helix and perturbs microtubule–kinesin interactions in yeast (Tischfield *et al.*, 2010). Thus, the neuropathy likely arises from a dominant mechanism that disrupts specific aspects of motor protein–based axonal transport.

The TUBB3 E410K substitution causes facial dysmorphisms

We can only speculate on the aetiology of TUBB3 E410K facial dysmorphisms. Although neural crest gives rise to facial cartilage

Table 5 Endocrine characteristics of individuals with TUBB3 E410K substitution

Proband	I	II	III	IV	V	VI	VII	VIII
Age (years)/gender (M/F)	7 F	9 M	9 F	15 F	19 F	20 M	21 M	25 M
Pre-treatment								
Age at assessment	5 years	11 months	13 weeks	15 years, 2 months	19 years	16 years, 9 months	19 years	16 years
External genitalia at birth	Normal female	Male	Normal female	Normal female	Normal female	Male	Male	Male
Micropenis		Micropenis				ND	Micropenis	ND
Testicular descent		Cryptorchidism				Normal	Cryptorchidism	Cryptorchidism
Pubertal status								
Testicular volume						**3 ml (pre-pubertal)**	**2 ml (pre-pubertal)**	ND
Thelarche stage	Tanner 1		Tanner 1	Tanner 2	Tanner 1			
Menarche	No		No	No	No			
Ovaries by US	ND		ND	ND	Hypoplastic			
Biochemistry								
LH (mIU/ml)	ND	ND	ND	**<1**	**<0.07**	**0.27**	**<0.1**	**<0.1**
FSH (mIU/ml)	ND	ND	ND	**<1**	**<0.07**	**0.34**	**0.45**	**0.26**
T (ng/dl)	ND	ND	ND			**<10**	**<10**	ND
E2 (pg/ml)	ND	ND	ND	**0.1**	**<10**			
Other results		T 20 ng/dl after hCG stimulation 1000 U × 3						
Post-treatment								
Age at reassessment		17 months				20 years, 2 months	21	22
Treatment and dose		50 mg IM monthly				50–100 mg IM monthly	50 mg IM monthly	50 mg IM monthly
Length of treatment		3 months				16 months	21 months	6 years
Penile length		4.5 cm				–	7 cm	–
Testicular descent		Cryptorchidism resolved				–	–	–
Pubertal status								
Testicular volume		ND				12 ml (post–pubertal)	**2 ml**	**5 ml**
Thelarche stage								
Menarche								

Abnormal values are in bold. 'Pre-treatment' and 'post-treatment' refer to testosterone replacement therapy.
M = male; F = female; E2 = oestradiol; FSH = follicle stimulating hormone; hCG = human chorionic gonadotropin; IM = intramuscularly; LH = luteinizing hormone; ND = no data; T = testosterone; US = ultrasound.

Table 6 Polymorphisms in Kallmann syndrome genes

Gene	Subject				
	II	IV	VI	VII	VIII
CHD7	c.6003C>T (p.A2001A) c.6135G>A (rs6999971)	c6103+8C>T (rs3763592) c.6276G>A (rs2068096)	c.6135G>A (rs6999971)	X	X
FGF8	X	X	X	X	X
FGFR1	X	X	X	X	X
KAL1	X	X	X	X	c.1600G>A (rs808119) c.1833C>T (rs809446)
NELF	X	X	X	X	X
PROK2	X	X	X	X	X
PROKR2	X	X	X	c.585G>C (rs3746682)	c.585G>C (rs3746682)

c. = codon; p. = protein amino acid; rs = dbSNP-assigned reference single nucleotide polymorphism identification number (http://www.ncbi.nlm.nih.gov/snp); X = no polymorphisms.

and bone, *TUBB3* is reportedly expressed only transiently in neural crest cells destined to become neurons (Haendel *et al.*, 1996), so this seems an unlikely aetiology. Although congenital muscle paralysis may alter skeletogenesis (Sharir *et al.*, 2011), the facial dysmorphology of subjects with the TUBB3 E410K substitution is dissimilar to that of subjects with facial weakness and absent facial nerve secondary to homozygous *HOXB1* mutations (Webb *et al.*, 2012). Given that affected individuals also have Kallmann syndrome, it is intriguing to consider that TUBB3 E410K could disturb nasal patterning by altering development of neural elements within the nasal placode, which normally secretes cues that influence the development of mesenchyme that gives rise to the nasal conchae and nasal bone (Szabo-Rogers *et al.*, 2009).

The TUBB3 E410K substitution produces a specific and consistent set of phenotypes that highlights the selective vulnerability of cell populations to altered TUBB3 function

Our previous report demonstrated that CFEOM-causative TUBB3 mutations alter axon guidance in the central and peripheral nervous system. Many of the findings we report here (selective cranial nerve abnormalities, Kallmann syndrome, peripheral neuropathy and intellectual disability associated with corpus callosum and anterior commissure defects in the absence of known cortical migration deficits) are consistent with abnormalities in both central and peripheral axon guidance and peripheral axon maintenance. It is more difficult to explain how the additional findings of stereotyped facial dysmorphisms and cyclic vomiting are the consequence of disrupted axon guidance or maintenance. The co-segregation of these two features with the other findings suggests that either undefined defects in axon guidance or maintenance contribute to their pathogenesis, or that they result from additional dysfunction of E410K mutant TUBB3 that is yet to be characterized.

The individuals that harbour the TUBB3 E410K substitution demonstrate a set of highly selective phenotypes that result from a single amino acid substitution in a protein expressed in all neurons, and thus highlight the vulnerability of specific populations of cells to alterations in TUBB3 function. The characteristic findings are also so distinctive that we believe clinicians will be able to identify affected individuals and predict the TUBB3 E410K substitution based on clinical features alone. This, in turn, will assist in distinguishing this population from those with typical Moebius syndrome and allow clinicians to provide a more accurate diagnostic and prognostic picture to patients and families. Further characterization of the TUBB3-related syndromes and studies of their associated mutations should continue to improve clinical diagnosis and provide additional insight into the role that TUBB3 plays in the development and maintenance of neurons and the mechanism by which changes in its function may cause disease.

Funding

National Eye Institute of the National Institutes of Health [R01EY12498 to E.C.E.]; the Boston Children's Hospital Intellectual and Developmental Disabilities Research Centre [HD018655 to S.L.P., E.C.E.]; the National Institute of Child Health & Human Development of the National Institutes of Health [U54 HD28138 and T32 HD07396 to W.F.C.]; Children's Hospital Ophthalmology Foundation [to E.C.E., D.G.H.]; the Moebius Syndrome Foundation [to E.C.E.]. S.C is funded by the HHMI Medical Fellowship and Harvard Medical School. E.C.E. is a Howard Hughes Medical Institute Investigator.

Acknowledgements

The authors would like to thank the study participants and their families for their generosity and time, and Drs. Max Tischfield, Joseph Mazjoub, Anna Minster, and Athos Bousvaros for helpful discussions.

References

Abdollahi MR, Morrison E, Sirey T, Molnar Z, Hayward BE, Carr IM, et al. Mutation of the variant alpha-tubulin TUBA8 results in polymicrogyria with optic nerve hypoplasia. Am J Hum Genet 2009; 85: 737–44.

Abid F, Hall R, Hudgson P, Weiser R. Moebius syndrome, peripheral neuropathy and hypogonadotrophic hypogonadism. J Neurol Sci 1978; 35: 309–15.

Balasubramanian R, Crowley WF Jr. Isolated GnRH deficiency: a disease model serving as a unique prism into the systems biology of the GnRH neuronal network. Mol Cell Endocrinol 2011; 346: 4–12.

Balasubramanian R, Dwyer A, Seminara SB, Pitteloud N, Kaiser UB, Crowley WF Jr. Human GnRH deficiency: a unique disease model to unravel the ontogeny of GnRH neurons. Neuroendocrinology 2010; 92: 81–99.

Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol 2009; 5: 569–76.

Boles RG, Powers AL, Adams K. Cyclic vomiting syndrome plus. J Child Neurol 2006; 21: 182–8.

Boles RG, Adams K, Ito M, Li BU. Maternal inheritance in cyclic vomiting syndrome with neuromuscular disease. Am J Med Genet A 2003; 120A: 474–82.

Brackett LE, Demers LM, Mamourian AC, Ellenberger C, Santen RJ. Moebius syndrome in association with hypogonadotropic hypogonadism. J Endocrinol Invest 1991; 14: 599–607.

CDC. Clinical Growth Charts. 2009 [cited 2012 April 18]; Available from: http://www.cdc.gov/growthcharts/clinical_charts.htm-Set2 (4 August 2009, date last accessed).

Chelimsky TC, Chelimsky GG. Autonomic abnormalities in cyclic vomiting syndrome. J Pediatr Gastroenterol Nutr 2007; 44: 326–30.

Crowley WF. The developmental biology of the GnRH neurons. Mol Cell Endocrinol 2011; 346: 1–3.

Demer JL, Clark RA, Tischfield MA, Engle EC. Evidence of an asymmetrical endophenotype in congenital fibrosis of extraocular muscles type 3 resulting from TUBB3 mutations. Invest Ophthalmol Vis Sci 2010; 51: 4600–11.

Ertekin V, Selimoglu MA, Altnkaynak S. Prevalence of cyclic vomiting syndrome in a sample of Turkish school children in an urban area. J Clin Gastroenterol 2006; 40: 896–8.

Fallet-Bianco C, Loeuillet L, Poirier K, Loget P, Chapon F, Pasquier L, et al. Neuropathological phenotype of a distinct form of lissencephaly associated with mutations in TUBA1A. Brain 2008; 131 (Pt 9): 2304–20.

Haendel MA, Bollinger KE, Baas PW. Cytoskeletal changes during neurogenesis in cultures of avain neural crest cells. J Neurocytol 1996; 25: 289–301.

Hall J, Froster-Iskenius U, Allanson J. Handbook of normal physical measurements. Oxford: Oxford University Press; 1989.

Jaglin XH, Poirier K, Saillour Y, Buhler E, Tian G, Bahi-Buisson N, et al. Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria. Nat Genet 2009; 41: 746–52.

Jennings JE, Costigan C, Reardon W. Moebius sequence and hypogonadotrophic hypogonadism. Am. J.Med Genet A 2003; 123A: 107–10.

Jiang YQ, Oblinger MM. Differential regulation of beta III and other tubulin genes during peripheral and central neuron development. J Cell Sci 1992; 103 (Pt 3): 643–51.

Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, Ogata T, Sato N, Claahsen-van der Grinten HL, et al. CHD7 mutations in patients initially diagnosed with Kallmann syndrome—the clinical overlap with CHARGE syndrome. Clin Genet 2009; 75: 65–71.

Kallmann F, Schoenfeld W, Barrera S. The genetic aspects of primary eunuchoidism. Am J Ment Defic 1944; 48: 203–36.

Katsetos CD, Legido A, Perentes E, Mork SJ. Class III beta-tubulin isotype: a key cytoskeletal protein at the crossroads of developmental neurobiology and tumor neuropathology. J Child Neurol 2003; 18: 851–66; discusion 867.

Kawai M, Momoi T, Fujii T, Nakano S, Itagaki Y, Mikawa H. The syndrome of Mobius sequence, peripheral neuropathy, and hypogonadotropic hypogonadism. Am. J. Med. Genet. 1990; 37: 578–82.

Keays DA, Tian G, Poirier K, Huang GJ, Siebold C, Cleak J, et al. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. Cell 2007; 128: 45–57.

Kumar RA, Pilz DT, Babatz TD, Cushion TD, Harvey K, Topf M, et al. TUBA1A mutations cause wide spectrum lissencephaly (smooth brain) and suggest that multiple neuronal migration pathways converge on alpha tubulins. Hum Mol Genet 2010; 19: 2817–27.

Li BU, Balint JP. Cyclic vomiting syndrome: evolution in our understanding of a brain-gut disorder. Adv Pediatr 2000; 47: 117–60.

Li BU, Lefevre F, Chelimsky GG, Boles RG, Nelson SP, Lewis DW, et al. North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition consensus statement on the diagnosis and management of cyclic vomiting syndrome. J Pediatr Gastroenterol Nutr 2008; 47: 379–93.

Moebius PJ. Ueber angeborene doppelseitige Abducens-Facialis-Lahmung. Münchener medizinische Wochenschrift 1888; 35: 91–94.

Olson WH, Bardin CW, Walsh GO, Engel WK. Moebius syndrome: lower motor neuron involvement and hypogonadotropic hypogonadism. Neurology 1970; 20: 1002–8.

Pitteloud N, Boepple PA, DeCruz S, Valkenburgh SB, Crowley WF Jr, Hayes FJ. The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab 2001; 86: 2470–5.

Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, et al. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2002; 87: 152–60.

Poirier K, Saillour Y, Bahi-Buisson N, Jaglin XH, Fallet-Bianco C, Nabbout R, et al. Mutations in the neuronal ss-tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. Hum Mol Genet 2010; 19: 4462–73.

Preston D, Shapiro B. Nerve conduction studies: normal adult values. Electromyography and neuromuscular diseases. 2nd edn. Philadelphia, PA: Elsevier; 2005. p. 663–5.

Rollins JD, Collins JS, Holden KR. United States head circumference growth reference charts: birth to 21 years. J Pediatr 2010; 156: 907–13, 913 e1–2.

Rubinstein AE, Lovelace RE, Behrens MM, Weisberg LA. Moebius syndrome in Kallmann syndrome. Arch Neurol 1975; 32: 480–2.

Salpietro CD, Briuglia S, Merlino MV, Di Bella C, Rigoli L. A mitochondrial DNA mutation (A3243G mtDNA) in a family with cyclic vomiting. Eur J Pediatr 2003; 162: 727–8.

Saul R, Geer J, Seaver L, Phelen M, Sweet K, Mills C. Growth references: third trimester to adulthood. Greenwood, South Carolina: Greenwood Genetic Center; 1998.

Schwanzel-Fukuda M. Origin and migration of luteinizing hormone-releasing hormone neurons in mammals. Microsc Res Tech 1999; 44: 2–10.

Sharir A, Stern T, Rot C, Shahar R, Zelzer E. Muscle force regulates bone shaping for optimal load-bearing capacity during embryogenesis. Development 2011; 138: 3247–59.

Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. Proc Natl Acad Sci USA 2010; 107: 15140–4.

Szabo-Rogers HL, Geetha-Loganathan P, Whiting CJ, Nimmagadda S, Fu K, Richman JM. Novel skeletogenic patterning roles for the olfactory pit. Development 2009; 136: 219–29.

Tache Y. Cyclic vomiting syndrome: the corticotropin-releasing-factor hypothesis. Dig Dis Sci 1999; 44 (8 Suppl): 79S–86S.

Tischfield MA, Baris HN, Wu C, Rudolph G, Van Maldergem L, He W, et al. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. Cell 2010; 140: 74–87.

Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, et al. Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic

hypogonadotrophic hypogonadism. Proc Natl Acad Sci USA 2011; 108: 11524–9.

Wang Q, Ito M, Adams K, Li BU, Klopstock T, Maslim A, et al. Mitochondrial DNA control region sequence variation in migraine headache and cyclic vomiting syndrome. Am J Med Genet A 2004; 131: 50–8.

Webb BD, Shaaban S, Gaspar H, Cunha LF, Schubert CR, Hao K, et al. HOXB1 founder mutation in humans recapitulates the phenotype of Hoxb1-/- mice. Am J Hum Genet 2012; 91: 171–9.

Zankl A, Eberle L, Molinari L, Schinzel A. Growth charts for nose length, nasal protrusion, and philtrum length from birth to 97 years. Am J Med Genet 2002; 111: 388–91.