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ORIGINAL INVESTIGATION

Expanding the clinical and mutational spectrum of Kaufman oculocerebrofacial syndrome with biallelic *UBE3B* mutations

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Abstract Biallelic mutations of *UBE3B* have recently been shown to cause Kaufman oculocerebrofacial syndrome (also reported as blepharophimosis–ptosis–intellectual disability syndrome), an autosomal recessive condition characterized by hypotonia, developmental delay, intellectual disability, congenital anomalies, characteristic facial dysmorphic features, and low cholesterol levels. To date, six patients with either missense mutations affecting the UBE3B HECT domain or truncating mutations have been described. Here, we report on the identification of homozygous or compound heterozygous *UBE3B* mutations in six additional patients from five unrelated families using either targeted *UBE3B* sequencing in individuals with suggestive facial dysmorphic features, or exome sequencing. Our results expand the clinical and mutational spectrum of the *UBE3B*-related disorder in several ways. First, we have identified *UBE3B* mutations in individuals who previously received distinct clinical diagnoses: two sibs with Toriello–Carey syndrome as well as the patient reported to have a "new" syndrome by Buntinx and Majewski in 1990. Second, we describe the adult phenotype and clinical variability of the syndrome. Third, we report on the first instance of homozygous missense alterations outside the HECT domain of UBE3B, observed in a patient with mildly dysmorphic facial features. We conclude that *UBE3B* mutations cause a clinically recognizable and possibly underdiagnosed syndrome characterized by distinct craniofacial

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features, hypotonia, failure to thrive, eye abnormalities, other congenital malformations, low cholesterol levels, and severe intellectual disability. We review the *UBE3B*-associated phenotypes, including forms that can mimick Toriello–Carey syndrome, and suggest the single designation "Kaufman oculocerebrofacial syndrome".

Introduction

Kaufman oculocerebrofacial syndrome (KOS; MIM 244450) is an autosomal recessive disorder characterized by developmental delay and intellectual disability (ID), microcephaly, hypotonia, structural eye anomalies and other organ malformations, as well as distinctive facial dysmorphic features. Fewer than 10 affected individuals had been reported from the first description in 1971 to 2012 (Kaufman et al. 1971; Figuera et al. 1993; Garcia-Cruz et al. 1988; Dentici et al. 2011) when we redescribed the syndrome (under the acronym BPID syndrome, blepharophimosis–ptosis–intellectual disability syndrome; MIM 615057) and identified biallelic *UBE3B* mutations as the underlying cause (Basel-Vanagaite et al. 2012). In that report, patients with BPID syndrome were thought to have a distinctive condition because they lacked the eye abnormalities characteristic to patients with KOS, such as microphthalmia and optic nerve anomalies. There is a phenotypic overlap between KOS, Toriello–Carey syndrome (TCS; agenesis of the corpus callosum with facial anomalies and Pierre-Robin sequence; MIM 217980; Toriello et al. 2003) and the phenotype reported by Buntinx and Majewski as a "new" syndrome in 1990 (Buntinx and Majewski 1990). The differential diagnosis of KOS and related syndromes extends to other blepharophimosis–intellectual disability syndromes (or Ohdo-like syndromes) such as Ohdo syndrome (MIM 249620), *KAT6B*-associated Say-Barber/Biesecker/Young-Simpson syndrome (MIM 603736; Clayton-Smith et al. 2011), *MED12*-associated Maat-Kievit-Brunner syndrome (MIM 300895; Vulto-van Silfhout et al. 2013), and Dubowitz syndrome (MIM 223370) (reviewed by Verloes et al. 2006).

Only six patients with KOS/BPID syndrome and biallelic *UBE3B* mutations have been described previously (Basel-Vanagaite et al. 2012; Flex et al. 2013). Here, we describe six additional patients and review the clinical and molecular spectrum of this recognizable entity.

Methods

Patient ascertainment and clinical evaluations

The study was approved by the Ethics Committees of the Universities of Ulm and Erlangen-Nürnberg, and written informed consent was obtained from the patients' parents or legal guardian. We report on six individuals from five unrelated families. The affected individuals were evaluated by routine medical history interviews and a physical examination, and clinical records were reviewed. All six patients had undergone brain imaging [magnetic resonance imaging (MRI) in patients 1-5 and computed tomography in patient 6], and the MRI scans were reviewed by one radiologist (N.B.). A basic endocrinological evaluation had been performed in all affected individuals.

In patients 1–3, KOS was not regarded as a differential diagnosis and mutations were identified following clinical diagnostic or research exome sequencing. After the reports on *UBE3B* mutations as the cause of KOS (Basel-Vanagaite et al. 2012; Flex et al. 2013) the attending geneticists of patients 1–3 contacted L.B.-V. and G.B. *UBE3B* mutations were suspected in patients 4–6 based mainly on facial dysmorphic features following a literature review performed by one of the authors (L.B.-V.) although these three children had previously received clinical diagnoses different from KOS/BPID. Patients 4 and 5 are siblings and have been reported with a diagnosis of typical TCS (Toriello et al. 2003); and patient 6 was reported by Buntinx and Majewski (1990).

Exome and Sanger sequencing

UBE3B mutations were identified by whole exome sequencing in patients 1–3 and by Sanger sequencing of the coding exons and exon–intron boundaries in patients 4–6. After the identification of a mutation in a sibling pair with a previous diagnosis of TCS, we sequenced *UBE3B* in four additional unrelated patients with TCS, two of whom have been reported previously (Toriello et al. 2003). Exome sequencing was performed using the SureSelect Human All Exon 50 Mb Kit (Agilent) and a paired-end protocol on an Illumina HiSeq 2000 sequencer (patients 1 and 3) or a SOLiD 5500xl instrument (patient 2). Reads were

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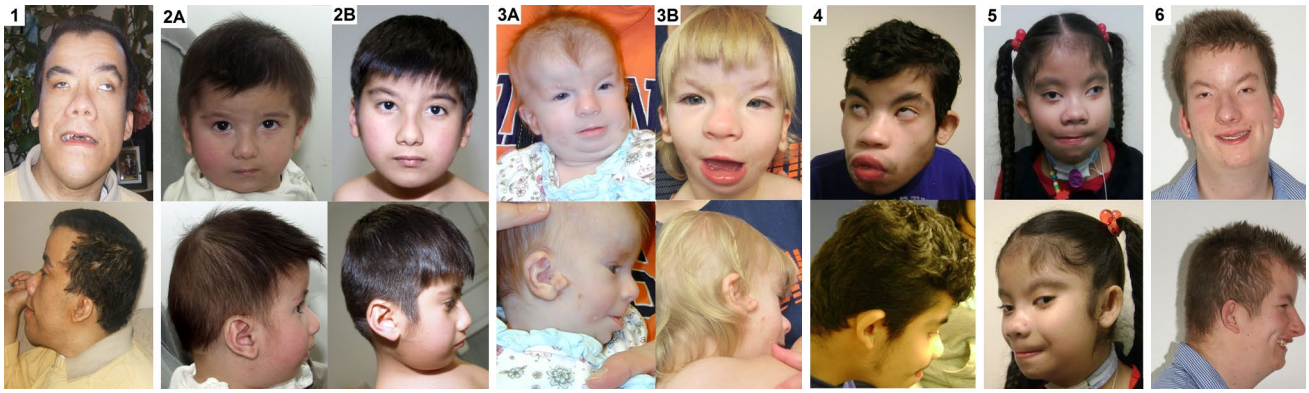

Fig. 1 Facial features of the individuals described in this study. The numbers correspond to the numbers in Table 1. Ages of the patients are as follows: *1*, 33 years; *2A*, 8 months; *2B*, 5 years 6 months; *3A*, 4 months; *3B*, 2 years; *4*, 16 years; *5*, 10 years 8 months; *6*, 25 years

aligned to the human genome assembly hg19 (GRCh37) and variants were called and annotated using software integrated in the respective in-house exome analysis pipeline as described previously [Gandomi et al. 2013, for patients 1 and 3; Hansen et al. 2013 (patient MR043), for patient 2]. This resulted in a mean coverage of 136, 97, and 92× in patients 1–3, respectively; and 91, 80, and 89 % of the target bases were covered 10× or more. In individuals born to consanguineous parents, we additionally performed a search for large regions of homozygosity, either by identifying runs of consecutive homozygous SNPs in exome sequencing data essentially as described previously (Basel-Vanagaite et al. 2012) or by genotyping patient DNA with Affymetrix Genome-wide Human SNP 6.0 arrays. PCR and Sanger sequencing were performed for the validation of variants of interest and for cosegregation studies.

In patients 4–6, the 26 *UBE3B* coding exons and respective exon–intron boundaries were sequenced on genomic DNA as described previously (Basel-Vanagaite et al. 2012). Potentially disease-causing variants were analyzed for their frequency by querying the NHLBI Exome Sequencing Project database (Exome Variant Server; http://evs.gs.washington.edu/EVS). In case of patient 2, we additionally sequenced the *UBE3B* exons of interest in 188 Turkish control individuals. Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) and SIFT (http://sift.jcvi.org) were used to predict the impact of missense alterations on protein function.

Results

Clinical reports and identification of *UBE3B* mutations

Facial features of patients 1–6 are shown in Fig. 1, clinical details are summarized in Table 1, and brain MRIs are shown in Supplementary Fig. 1.

Patient 1 (Fig. 1, picture 1; Table 1) is a 33-year-old man with profound ID and no speech. He is the second son (of three) born to unaffected parents of Hispanic ancestry who are second cousins once removed. He presented at birth with muscular hypotonia and hearing impairment. Unlike the other patients in whom microcephaly or an occipitofrontal circumference (OFC) <10th centile was observed, his OFC was at the 75th percentile. Relative macrocephaly was probably of familial origin as his father was reported to be macrocephalic. Brain MRI showed moderately dilated lateral ventricles in patient 1 (Supplementary Fig. 1) and diffuse anomalies of the white matter frontally. He has an abnormal sleep pattern and occasional violent behavior.

Using clinical diagnostic whole-exome sequencing, we detected a homozygous nonsense mutation in *UBE3B*, c.61G>T (p.Glu21*) (Table 2; Supplementary Fig. 2). The patient's mother and unaffected brother were heterozygous carriers of the mutation, as shown by Sanger sequencing; no DNA from the deceased father was available for genetic studies. In addition, alterations in two other ID-related genes were identified by exome sequencing and confirmed by Sanger sequencing; namely, a rare homozygous missense alteration affecting a highly conserved amino acid, c.878G>A (p.Arg293Gln; rs35267264), in *KIAA1033*, and a heterozygous missense alteration of a highly conserved amino acid, c.1307C>T (p.Pro436Leu), in *EHMT1*. A single homozygous *KIAA1033* mutation has previously been suggested as the cause of moderate to severe ID, poor language and adaptive skills, delayed fine motor development, and short stature in a family from Oman (Ropers et al. 2011). Heterozygous alterations in *EHMT1* cause Kleefstra syndrome, which is characterized by severe ID, hypotonia, brachycephaly, flat face with hypertelorism, upslanting palpebral fissures and a thick everted lower lip (Kleefstra et al. 2006). The following evidence supports the homozygous *UBE3B* nonsense mutation, rather than one of the variants in *KIAA1033* or *EHMT1*, as the major contributor to the

Table 1 Clinical findings in 12 individuals with biallelic *UBE3B* mutations

	Family 1	Family 2	Family 3	Family 4		Family 5	Family 6	Family 7		Family 8	Family 9	Family 10	Total
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	
Reference	This report	This report	This report	(Toriello et al. 2003)	(Toriello et al. 2003)	(Buntinx and Majewski 1990)	(Basel-Vanagaite et al. 2012)	(Basel-Vanagaite et al. 2012)	(Basel-Vanagaite et al. 2012)	(Basel-Vanagaite et al. 2012)	(Flex et al. 2013)	(Flex et al. 2013)	
Age at last examination	33 years	7 years 5 months	24 months	16 years	10 years 8 months	25 years	3 years 3 months	7 years 7 months	3 years 1 months	1 year	NR	NR	
Origin	Hispanic	Turkish	German and Irish	Mexican	Mexican	German	Israeli Arab	Italian	Italian	Tunisian	Italian	Italian	
Parental consanguinity	+	+	–	Possibly remote	Possibly remote	–	+	–	–	+	+	–	
Sex	M	M	F	M	F	M	F	F	M	F	F	F	
Growth													
Short stature	+	–	–	+	+	+	–	+	+	+	NR	NR	7/10
Microcephaly/OFC <10th centile	–	+	+	+	+	+	+	+	+	+	+	+	11/12
Underweight	–	–	+	+	+	NR	+	+	+	NR	NR	NR	6/8
Skin and hair													
Sparse thin scalp hair	–	–	–	+	+	–	+	+	+	+	–	–	6/12
Thin skin	–	–	–	–	–	–	+	+	+	+	+	+	6/12
Craniofacial													
Long face	+	–	–	+	–	–	–	+	+	+	+	–	6/12
Arched/ sparse laterally broad eyebrows	+	–	+	+	+	+	+	+	+	+	+	+	11/12
Upward slanting palpebral fissures	+	–	–	+	+	+	–	+	+	–	+	+	8/12
Epicanthal folds/ telecanthus	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Blepharophimosis with or without ptosis	+	–	+	+	+	+	+	+	+	+	+	+	11/12
Flat zygomata	+	+	–	–	–	+	–	+	+	+	+	+	8/12
Short nose	+	–	–	+	+	+	+	+	+	+	+	+	10/12
Anteverted nares	+	–	+	+	+	+	+	+	+	+	+	+	11/12
Small mouth	+	+	–	+	+	+	+	+	+	+	+	+	11/12
Thin upper lip with or without absent cupid's bow	+	–	+	+ (thick lips later in life)	+	+	–	+	+	+	+	–	9/12
Micro/retrognathia	–	+	+	+	+	+	+	+	+	+	+	+	11/12

Table 1 continued

	Family 1	Family 2	Family 3	Family 4		Family 5	Family 6	Family 7		Family 8	Family 9	Family 10	Total
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	
Dysplastic ears	+	+	+ (with preauricular tags)	+	+	+	+	+	+	+	+ (with preauricular tags)	+ (with preauricular tags)	12/12
Palatal anomalies	–	+	–	+	–	+	+	+	–	–	+	+	7/12
Respiratory system													
Tracheomalacia/laryngomalacia/stridor	–	–	–	+	+	–	+	–	–	+	+	+	6/12
Cardiovascular system													
Congenital heart disease	–	–	–	–	+ (mild distal right pulmonary artery stenosis and septal hypertrophy)	–	+ (atrial septal defect, ventricular septal defect, aortic coarctation)	–	–	+ (atrial septal defect)	–	–	3/12
Gastrointestinal system													
Feeding difficulties in infancy/childhood	NR	+	+	+	+	+	+	+	+	+	+	+	11/11
Constipation	–	–	–	+	–	–	–	+	+	–	+	+	5/12
Urogenital system													
Genital abnormalities	–	–	–	+ (small penis)	+ (hypoplastic labia majora)	–	–	–	–	+ (hypoplastic labia majora)	+ (Clitoromegaly)	+ (Clitoromegaly)	5/12
Renal abnormalities	–	–	+ (abnormal shape of one kidney without functional problem)	–	–	+ (grade V vesicoureteral reflux)	+ (mild left pyelectasis)	+ (double right kidney, right pyelectasis)	–	–	–	–	4/12
Skeletal system													
Structural finger/toe abnormalities	–	–	–	+	+	+	–	+	+	–	–	–	5/12
Pes talus varus/valgus	–	–	+ (metatarsus adductus)	–	+	–	–	–	–	–	+	+	4/12
Congenital dislocation of the hip/coxa valga	–	–	–	–	–	–	+	–	–	+	+	–	3/12

Table 1 continued

	Family 1	Family 2	Family 3	Family 4		Family 5	Family 6	Family 7		Family 8	Family 9	Family 10	Total
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	
Ocular													
Microcornea or microphthalmia	+	–	–	–	–	–	–	–	–	–	+	+	3/12
Strabismus	–	+	–	–	+	+	–	+	–	–	+	+	6/12
Iris coloboma	–	–	–	+	+	+	–	–	–	–	–	–	3/12
Myopia/astigmatism/hyperopia	–	–	+ mild myopia	+	+	+	+	+	–	–	+	–	7/12
Hearing													
Hearing impairment	+	+	+	+ (stenotic external auditory canals)	–	+	+	–	–	–	+	+	8/12
Neurological and brain imaging													
Seizures	–	–	–	+	+	+	–	–	–	+	+	+	6/12
Hypotonia/delayed motor milestones	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Anomalies on brain imaging	Dilatation of the lateral ventricles, anomalies of the frontal white matter	Dilatation of the lateral ventricles, thin corpus callosum	Short corpus callosum, frontal midline mass (lipoma?)	– (Normal brain MRI)	Dilatation of the lateral ventricles; Chiari type 1 malformation	Agenesis of the corpus callosum	Dilatation of the lateral ventricles, Chiari type 1 malformation, hypoplastic corpus callosum	Agenesis of the rostrum of the corpus callosum	Reduced size of the pituitary gland, partial empty sella	Hypoplastic corpus callosum	NR	NR	9/10
Cholesterol levels													
Abnormalities of cholesterol levels	+	–	+	–	–	+	+	+	–	+	–	+	7/12
Cognitive development													
Absent speech	+	–	+	+	+	–	+	+	+	+	+	+	10/12
Developmental delay/intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+	12/12

NR not reported

patient's phenotype: (1) His clinical presentation and facial dysmorphism are similar to those observed in other patients with KOS. (2) The *KIAA1033* variant has a minor allele frequency of 0.14 % (NHLBI Exome Variant Server), suggesting that it may be a rare polymorphism. Notably, *KIAA1033* is located on chromosome 12q23.3, approximately 4.5 Mb away from *UBE3B*, and both genes are within a 46.6 Mb region of homozygosity on chromosome 12 in patient 1, as determined by homozygosity analysis in the exome sequence. (3) Patient 1 has no facial features suggestive of Kleefstra syndrome. Thus, although more than one of the three variants may contribute to the phenotype, including to clinical presentations that are atypical for KOS, the truncating *UBE3B* mutation is likely the major causative allele.

Patient 2 is a 7-year 5-month-old boy who presented with muscular hypotonia, congenital microcephaly and variable other anomalies (Fig. 1, pictures 2A and 2B; Table 1). He developed minimal speech (5–10 single words at 7 years of age) and has an estimated IQ of 20–49. His unaffected parents are first cousins originating from Turkey. Craniofacial dysmorphisms are mild, and he is the only patient in this study without blepharophimosis.

Whole-exome sequencing revealed two homozygous *UBE3B* missense mutations, c.1445T>A (p.Leu482His) and c.1616T>C (p.Leu539Pro; Table 2, Supplementary Fig. 2), with both parents being heterozygous for both mutations. *UBE3B* is located in a 21.75 Mb region of homozygosity in this patient's exome. Both mutations affect conserved amino acid residues located outside the IQ and HECT domains (Gong et al. 2003). Therefore, the relatively mild craniofacial phenotype in this patient might be related to the atypical location of the mutations compared to other patients, in whom missense alterations within the HECT domain are predicted to have severe consequences. In silico analysis revealed that Leu482 is located in a leucine-rich region spanning residues 472–500. The regular spacing of hydrophobic residues (every 7th amino acid position) resembles that of a leucine zipper (Hirst et al. 1996). Such leucine zippers form dimers, suggesting that this UBE3B region might be involved in intermolecular interactions. A similar function can be assigned to the region around Leu539, which is predicted to exhibit a coiled-coil structure (residues 512–560). For both mutation sites, the type of amino acid exchange will drastically affect the biophysical properties and is therefore likely to disrupt the respective protein-interaction motif.

Five other rare homozygous coding variants were confirmed by Sanger sequencing (none affecting a known disease-associated gene; data not shown).

Patient 3 (Fig. 1, pictures 3A and 3B; Table 1) is a 24-month-old girl. She has congenital microcephaly (currently her OFC is at −4 SD), bilateral large complex preauricular tags and stenotic external ear canals associated with moderate-to-severe conductive hearing impairment. She has significant feeding problems with gastroesophageal reflux and vomiting, requiring the placement of a gastrostomy tube (G-tube). She had two patches of hair on each cheek and hypertrichosis of the back. MRI scan showed partial agenesis of the corpus callosum and a frontal midline mass, thought to be a lipoma (Supplementary Fig. 1).

Clinical diagnostic whole exome sequencing revealed a homozygous missense mutation, c.2990G>C (p.Arg997Pro), in *UBE3B* (Table 2), affecting the HECT domain. The mutation was confirmed by Sanger sequencing (Supplementary Fig. 2), and the parents were heterozygous carriers of the mutation.

Patients 4 and 5 are siblings of Hispanic American origin with a clinical diagnosis of TCS (Fig. 3 and Fig. 2, respectively, in Toriello et al. 2003; Table 1). Recent pictures showing their facial dysmorphic features are displayed in Fig. 1, pictures 4 and 5. The presence of *UBE3B* mutations was suspected after reviewing relevant scientific publications and noticing suggestive facial features (Toriello et al. 2003).

Patient 4 is the older sibling and is currently 16 years old. From birth he has had severe respiratory and feeding problems with abnormal swallowing, which necessitated a Nissen fundoplication with G-tube placement at 2 months. At age 16, he is still G-tube dependent. He required a tracheostomy because of laryngomalacia and subglottic stenosis and underwent laryngotracheal reconstruction at 2 years 6 months of age. At the age of 13 years, he was found to have scoliosis and focal progressive kyphosis requiring surgical intervention.

Patient 5 is the 11-year-old younger sister of patient 4. She had a similar clinical presentation to that of her brother, including poor suck and severe respiratory problems. She also needed a tracheostomy because of tracheomalacia and is still tracheostomy dependent at age 11. She had significant aspiration and obstructive sleep apnea. She also underwent Nissen fundoplication and was fed through a G-tube. In addition, she underwent bilateral tarsometatarsal release and left cuboid closing wedge osteotomy. Both sibs showed general hypertrichosis in their first year of life.

Although their parents were not aware of consanguinity, SNP-array genotyping detected two large (>10 Mb) blocks of homozygosity in each of the siblings, possibly indicating remote parental consanguinity. The single region of shared homozygosity was a 11.4 Mb segment on chromosome 12q23.3–q24.22 that contained *UBE3B*. Sequencing of *UBE3B* revealed a homozygous c.2335G>A missense alteration in both affected sibs (p.Gly779Arg; Table 2, Supplementary Fig. 2); the parents were heterozygous carriers of the mutation. Gly779 is a highly conserved amino acid of the HECT domain.

We next sequenced *UBE3B* in four additional patients with TCS, none of whom had the characteristic facial appearance associated with KOS. We did not detect any mutations.

Patient 6. This man (Fig. 1, picture 6; Table 1) is currently 25 years old and the presence of a *UBE3B* mutation was suspected because of the recognition of characteristic facial features from a previously published report (Buntinx and Majewski 1990). The neonatal period was complicated by poor suck and hypotonia. Postaxial polydactyly of both hands was noticed at birth and treated by surgery. Currently, he has about 100 words and can speak in 3–4 word sentences.

Sequencing of *UBE3B* revealed a compound heterozygous mutation with a c.2098C>T (p.Gln700*) nonsense mutation inherited from his mother and a c.2990G>C (p.Arg997Pro) mutation (Table 2, Supplementary Fig. 2), which was also present in his unaffected brother and likely inherited from their deceased father.

Review of the clinical and laboratory features of patients with biallelic *UBE3B* mutations

Table 1 summarizes clinical findings on the patients with biallelic *UBE3B* mutations reported here and previously (Basel-Vanagaite et al. 2012; Flex et al. 2013).

Demographic data

Of the 12 patients with *UBE3B* mutations, seven were female. Consanguinity was reported in five of the families. In two families, there were two affected sibs.

Pregnancy and birth

No abnormal findings were reported following prenatal ultrasound except for polyhydramnion in one case and oligohydramnion in one case. All the patients for whom data were available were born at term.

Growth

Birth weight was normal in most of the patients. The majority of patients had an OFC below the 10th centile at birth. Most affected children had failure to thrive and postnatally decreasing age-matched OFC centiles. All but three affected individuals had short stature, and all but one had microcephaly or an OFC below the 10th centile.

Ectodermal abnormalities

Sparse scalp hair was described in infancy in 6/12 patients. The appearance of the scalp hair improved with age. The skin was described as thin in six patients. Three affected individuals had hypertrichosis, and two had eczema.

Craniofacial abnormalities

Several patients were described as having a flat occiput or brachycephaly. Characteristic craniofacial features included arched, sparse and laterally flared eyebrows,

Table 2 *UBE3B* mutations

Patient	Origin	Parental consanguinity	Mutation (DNA)	Predicted effect on protein	Protein domain	Control alleles (EVS)	Polyphen-2	SIFT
1	Hispanic American	Second cousins once removed	c.61G>T homozygous	p.(Glu21*)	Truncation N-terminal to IQ and HECT domains	0/13,006	n.a.	n.a.
2	Turkish	First cousins	c.1445T>A homozygous	p.(Leu482His)	Leucine-rich region	0/13,006 0/376 Turkish control alleles	Probably damaging	Not tolerated
			c.1616T>C homozygous	p.(Leu539Pro)	Predicted coiled-coil domain	0/13,006 0/376 Turkish control alleles	Probably damaging	Not tolerated
3	German and Irish	No	c.2990G>C homozygous	p.(Arg997Pro)	HECT domain	0/13,006	Probably damaging	Not tolerated
4 and 5	Mexican	No	c.2335G>A homozygous	p.(Gly779Arg)	HECT domain	0/13,006	Probably damaging	Not tolerated
6	German	No	c.2098C>T heterozygous	p.(Gln700*)	Truncation N-terminal to HECT domain	0/13,006	n.a.	n.a.
			c.2990G>C heterozygous	p.(Arg997Pro)	HECT domain	0/13,006	Probably damaging	Not tolerated

blepharophimosis with epicanthal folds with or without ptosis, flat zygomata, a characteristic nasal shape with a relatively narrow nasal bridge and broad nasal base, a flat philtrum (long or short), and thin lips, especially the upper lip, with absent cupid's bow, as well as a small mouth. The ears were often apparently low-set with over-folded helices or cupping, small earlobes and, in some instances, underdeveloped ear crus. Three patients had preauricular tags and two had stenotic external ear canals. With advancing age, the shape of the face became more elongated, the palpebral fissures became up-slanting and, in some patients the lip vermilion became more everted. One adult had a long chin and very thick alae nasi with narrow nares.

Respiratory tract abnormalities

Six patients had breathing problems including tracheomalacia, subglottic stenosis, laryngomalacia or stridor. Two children needed a tracheostomy. Two patients had a history of apnea either shortly after birth or obstructive sleep apnea later in life.

Cardiovascular system

Congenital heart malformations are not a common finding in the *UBE3B*-related syndrome (3/12 patients). Heart defects included pulmonary artery stenosis, ASD, VSD, and aortic coarctation.

Gastrointestinal tract abnormalities

Eleven patients had feeding difficulties including poor suck and gastroesophageal reflux. Three patients required G-tube feeding. Constipation was present in five individuals.

Urogenital abnormalities

Genital abnormalities were more frequent in females (although the difference is not statistically significant in this small series) and included hypoplastic labia majora and/or minora or clitoromegaly (4/6 females). Micropenis was described in 1/5 males. Renal abnormalities included vesicoureteral reflux up to grade V and duplicated renal pelvis.

Skeletal abnormalities

Skeletal abnormalities were observed in 10/12 individuals and included abnormal chest shape (bell-shaped thorax, pectus carinatum) and long and slender fingers and toes. Clinodactyly of the fifth finger and hypoplastic distal phalanges were also reported. Congenital dysplasia of the hip was diagnosed in two newborns. Uncommon skeletal findings included polydactyly, coxa valga, scoliosis, congenital arthrogryposis, and metatarsus adductus.

Ocular abnormalities

Three of the 12 patients had microcornea or microphthalmia, 6/12 strabismus, three coloboma of the iris, five myopia with or without astigmatism, one hyperopia, three a pale optic disc, and two entropion.

Hearing loss

Eight patients had hearing impairment, mostly conductive, but three individuals had sensorineural or mixed conductive–sensorineural hearing loss; and two had cholesteatoma.

Neurological abnormalities

Hypotonia and delayed motor milestones were observed in all affected individuals. Six patients had seizures, often fever related. Independent walking was mostly achieved by 4–5 years of age; the gait was frequently described as unsteady. ID was present in all patients and was described as severe to profound. Only two patients developed speech, which in both cases was limited. Structural CNS abnormalities included absent or hypoplastic corpus callosum in six patients and a Chiari 1 malformation in two (Supplementary Fig. 1).

Endocrine and cholesterol abnormalities

Elevated TSH and reduced thyroid gland volume were diagnosed in one and low GH and ACTH in another patient. Seven individuals had low cholesterol levels including low total cholesterol, low HDL, low LDL levels, or combinations thereof.

Discussion

Recognition of KOS should be possible on clinical grounds in many cases. A consistent and recognizable pattern of typical craniofacial features combined with hypotonia, failure to thrive, eye abnormalities, other congenital malformations, low cholesterol levels and severe ID should indeed facilitate early diagnosis of patients with *UBE3B* mutations. We note, however, that neither ocular nor cerebral malformations are mandatory findings in this "oculocerebrofacial" syndrome. Evaluation of individuals with KOS should involve all body systems including assessment of the respiratory and gastrointestinal tracts, cardiologic and ophthalmologic evaluation, hearing tests, renal ultrasound, investigation of thyroid function and cholesterol levels,

developmental assessment and orthopedic evaluation. This series also illustrates the facial dysmorphism in adults with KOS and underscores the phenotypic variability, e.g., with respect to speech or limb involvement. Although mild limb abnormalities such as long and slender fingers, clinodactyly and camptodactyly have been described in patients with *UBE3B* mutations, polydactyly has not been reported previously. While polydactyly may be a coincidental finding, a patient with facial features reminiscent of *UBE3B*-related features and polydactyly has been described by Gabrielli et al. (1994) as having a new form of oro-facio-digital syndrome. Other cases with suggestive clinical features—but no *UBE3B* sequence analysis yet—have been reported, such as the patient described by Al Frayh and Haque (1987).

It is of interest that three patients, two described in this series and one in our previous study (patient 4 in Basel-Vanagaite et al. 2012, who is listed as patient 10 in our Table 1), were diagnosed as having TCS. Although patients with *UBE3B* mutations and patients originally described by Toriello and Carey (1988) share several overlapping clinical features (short palpebral fissures, small nose with anteverted nares, abnormal ears, laryngeal anomalies, cardiac defects, agenesis of the corpus callosum, microcephaly, micrognathia and cleft palate), the facial gestalt still is not completely similar. We note that the facial dysmorphic features of children with *UBE3B* mutations previously thought to have TCS are more similar to KOS than to the disorder originally described by Toriello and Carey (1988). Thus, *UBE3B* mutations define KOS which includes BPID syndrome, the phenotype described by Buntinx and Majewski and a subset of patients previously diagnosed with TCS. Because Kaufman first described the typical clinical picture in 1971, we suggest—to avoid confusion—designating the syndrome with biallelic *UBE3B* mutations as "Kaufman oculocerebrofacial syndrome".

Apart from providing data expanding the clinical phenotype of KOS, our report illustrates some of the challenges and opportunities of exome sequencing in a clinical context. In patient 1, clinical exome sequencing detected rare variants in three ID-related genes. Whereas the clinical similarities with KOS highlighted the *UBE3B* mutation as the most relevant variant, we cannot exclude a contribution of the variants in *KIAA1033* and *EHMT1* to the phenotype. Large-scale studies have highlighted the difficulties in assigning a single variant to a patient's phenotype, and in rare instances exome sequencing might even confirm two genetic diseases in one individual (Yang et al. 2013), and such situations probably make it difficult in some cases to assign a diagnosis on clinical grounds only. Furthermore, our data also illustrate how exome sequencing can help to establish a diagnosis in patients with a mild or atypical disease presentation and thereby contribute to the delineation of the phenotypic spectrum of rare genetic disorders. In patients 2 and 3, respectively, the mild and atypical facial dysmorphism had not raised the suspicion of a *UBE3B*-related disorder, but exome sequencing revealed a presumably causative homozygous missense variant in each case. The identification of missense alterations outside the HECT domain in patient 2 who presented with mild craniofacial dysmorphism with no blepharophimosis suggests a possible genotype–phenotype correlation. Future studies will establish whether missense changes outside the HECT domain generally lead to a milder phenotype.

In conclusion, we report on six individuals with biallelic *UBE3B* mutations, thereby doubling the number of reported patients. Identification of *UBE3B* mutations has merged phenotypes that were previously suspected to represent distinct syndromes into a single, possibly underdiagnosed disorder which can be clinically recognized.

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