

## Supplementary Material

### LOSS OF FUNCTION OF THE E3 UBIQUITIN-PROTEIN LIGASE *UBE3B* CAUSES KAUFMAN OCULOCEREBROFACIAL SYNDROME

Elisabetta Flex,<sup>1</sup> Andrea Ciolfi,<sup>1,2</sup> Viviana Caputo,<sup>3</sup> Valentina Fodale,<sup>1</sup> Chiara Leoni,<sup>4</sup> Daniela Melis,<sup>5</sup>  
Maria Francesca Bedeschi,<sup>6</sup> Laura Mazzanti,<sup>7</sup> Antonio Pizzuti,<sup>3</sup> Marco Tartaglia<sup>1,8</sup> and  
Giuseppe Zampino<sup>4,8</sup>

<sup>1</sup>Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Rome, 00161, Italy. <sup>2</sup>Dipartimento di Medicina Molecolare, Università “La Sapienza”, Rome, 00185, Italy. <sup>3</sup>Dipartimento di Medicina Sperimentale, Università “La Sapienza”, Rome, 00185, Italy. <sup>4</sup>Istituto di Clinica Pediatrica, Università Cattolica del Sacro Cuore, Rome, 00168, Italy. <sup>5</sup>Dipartimento di Pediatria, Facoltà di Medicina e Chirurgia, Università “Federico II”, Naples, 80131, Italy. <sup>6</sup>UOD Genetica Medica, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. <sup>7</sup>Dipartimento di Pediatria, Università degli Studi di Bologna, 40138 Bologna, Italy. <sup>8</sup>These authors contributed equally to this project.

**Supplementary Table 1.** Clinical presentation of patients with Kaufman oculocerebrofacial syndrome.

**Supplementary Table 2.** Clinical presentation of patients with features partially overlapping with Kaufman oculocerebrofacial syndrome screened for *UBE3B* gene mutations.

**Supplementary Table 3.** Primer pairs and annealing temperatures used to amplify the *UBE3B* coding sequence, and sizes of PCR products.

**Supplementary table 1.** Clinical presentation of patients with Kaufman oculocerebrofacial syndrome.

Patient(s) <i>Reference</i>	UCSC-KS01 <i>this study</i>	UCSC-KS02 <i>this study</i>	4 cases (1)	1 case (2)	1 case (3)	2 cases (4)
<i>UBE3B</i> mutation status	c.556C>T <sup>1</sup> p.Arg186stop	c.1166G>A <sup>1</sup> p.Trp389stop	unknown	unknown	unknown	unknown
Sporadic/Familial Sex	sporadic female	sporadic female	familial 2 female, 2 male	sporadic female	sporadic male	sporadic female
Neonatal findings						
Weight	25 <sup>th</sup> cent.	3 <sup>rd</sup> cent.	variable <sup>2</sup>	25 <sup>th</sup> cent.	75 <sup>th</sup> cent.	3 <sup>rd</sup> cent.
Respiratory distress	+	+	+ (3/3)	+	+	+ (2/2)
Laryngeal stridor/ noisy breathing	+	+	+ (2/2)	n.r.	n.r.	+ (1/2)
Feeding difficulties	+	+	+ (3/4)	+	+	n.r.
Growth retardation	+	+	- (3/3)	-	+	+ (2/2)
Delayed psychomotor development	+	+	+ (4/4)	+	+	+ (2/2)
Delayed language	+	+	+ (4/4)	n.r.	n.r.	n.r.
Seizures	+	+	- (1/1)	n.r.	n.r.	n.r.
Shuffling gait	+	+	+ (3/4)	n.r.	n.r.	n.r.
Hypotonia	+	+	+ (2/3)	+	n.r.	+ (2/2)
Craniofacial features						
Microbrachycephaly	+	+	+ (3/3)	+	+	+ (2/2)
Long face	+	-	+ (3/3)	-	+	+ (1/2)
Sparse and laterally broad eyebrows	+	+	+ (3/3)	+	+	+ (2/2)
Upslanting palpebral fissures	+	+	+ (3/3)	+	+	+ (2/2)
Telecanthus	+	+	+ (1/2)	+	+	+ (2/2)
Blepharophimosis	+	+	- (1/1)	n.r.	+	+ (2/2)
Ptoxis	-	+	+ (1/1)	+	n.r.	-
Epicanthal folds	+	+	- (3/3)	+	+	+ (2/2)
Short nose	+	+	+ (1/2)	n.r.	n.r.	+ (1/1)
Anteverted nares/ columella below alae nasi	+	+	+ (1/2)	n.r.	n.r.	+ (1/1)
Long philtrum	+	+	+ (1/2)	+	+	+ (2/2)
Thin lips	+	-	+ (3/3)	+	+	+ (2/2)
Small mouth	+	+	+ (2/3)	n.r.	n.r.	+ (1/2)
Micrognathia	+	+	+ (4/4)	+	+	+ (2/2)
High, narrow palate	+	+	+ (1/1)	+	+	+ (2/2)
Small/frail teeth	+	+	+ (3/3)	+	-	+ (2/2)
Cup shaped ears	+	+	+ (4/4)	+	+	+ (1/2)
Preauricular tags	+	+	+ (4/4)	-	+	+ (1/2)
Auricular stenosis	+	+	n.r.	n.r.	n.r.	n.r.

**Suppl. Table 1. Continued**

Patient(s) <i>Reference</i>	UCSC-KS01 <i>this study</i>	UCSC-KS02 <i>this study</i>	4 cases (1)	1 case (2)	1 case (3)	2 cases (4)
Eye anomalies						
Microcornea	+	+	+ (3/3)	+	-	+ (1/2)
Strabismus	+	+	+ (3/3)	+	-	+ (2/2)
Pale disk	+	+	+ (3/3)	+	+	- (2/2)
Nystagmus	-	-	+ (1/2)	-	-	- (2/2)
Myopia	+	-	+ (3/3)	+	n.r.	+ (1/2)
Hearing impairment	+	+	n.r.	n.r.	n.r.	n.r.
Skeletal features						
Bell shaped thorax	+	+	+ (2/2)	-	+	+ (2/2)
Long and thin fingers	+	+	+ (2/2)	+	+	+ (2/2)
Coxa valga	+	-	+ (2/2)	n.r.	n.r.	n.r.
Pes talus valgus/varus	+	+	n.r.	n.r.	+	+ (1/2)
Clitoromegaly	+	+	n.r.	+	+	+ (1/2)
Constipation	+	+	n.r.	+	+	+ (1/2)
Gastroesophageal reflux	+	+	n.r.	+ <sup>3</sup>	n.r.	n.r.
Thin skin	+	+	n.r.	n.r.	n.r.	n.r.
Reduced cholesterol levels	-	+ <sup>4</sup>	n.r.	n.r.	n.r.	n.r.

<sup>1</sup>Homozygous change by descent.

<sup>2</sup>3<sup>rd</sup> cent. (N=1), 10<sup>th</sup> cent. (N=1), 75<sup>th</sup> cent. (N=2).

<sup>3</sup>Pyloric spasm.

<sup>4</sup>Low total/HDL cholesterol.

n.r., not reported.

**Supplementary Table 2.** Clinical presentation of patients with features partially overlapping with Kaufman oculocerebrofacial syndrome and negative for mutations in the coding sequence of *UBE3B*.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13 <sup>1</sup>
Sex	m	m	f	f	f	m	m	f	m	f	m	f	f
Neonatal findings													
Respiratory distress	-	-	+	-	+	-	-	-	-	-	n.r.	+	+
Laryngeal stridor/ noisy breathing	-	-	-	-	-	-	-	-	-	-	n.r.	-	-
Feeding difficulties	-	+	-	-	-	-	-	-	-	-	n.r.	-	-
Growth retardation	+	+	-	-	+	+	-	+	n.r.	n.r.	n.r.	n.r.	+
Delayed psychomotor development	-	+	+	+	+	-	-	+	-	+	-	+	+
Delayed language	-	+	-	+	+	-	-	+	-	+	-	+	+
Seizures	-	+	-	-	-	-	-	-	-	-	-	-	+
Shuffling gait	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypotonia	-	+	-	+	+	-	n.r.	+	-	-	-	-	-
Craniofacial features													
Microcephaly	-	+	-	-	+	-	-	+	+	-	-	+	+
Long face	+	-	-	+	+	-	-	-	-	-	-	-	+
Sparse and laterally broad eyebrows	-	+	-	+	+	-	-	+	-	-	-	+	+
Upslanting palpebral fissures	-	-	-	-	-	-	+	-	-	-	+	+	+
Telecanthus	+	-	+	-	-	-	+	-	-	-	-	-	+
Blepharophimosis	+	+	+	+	+	-	-	+	+	+	+	+	-
Ptosis	-	+	-	-	+	-	-	-	-	-	-	-	+
Epicanthal folds	-	+	-	+	+	-	+	+	-	-	-	-	-
Short nose	-	+	+	+	+	-	-	+	-	-	-	-	-
Anteverted nares/ columella below alae nasi	+	+	-	+	+	-	-	+	-	-	-	+	+
Long philtrum	-	+	-	+	+	-	-	+	-	-	-	-	+
Thin lips	-	-	-	+	+	-	-	+	-	-	-	-	+
Small mouth	n.r.	+	-	-	-	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	-
Micrognathia	+	+	-	+	-	-	-	+	-	-	-	-	+
High, narrow palate	+	+	-	+	-	-	-	+	-	-	-	-	+
Small/frail teeth	-	+	-	-	-	-	-	-	-	-	-	n.r.	-
Cup shaped ears	+	+	-	+	-	-	-	+	+	-	+	-	-
Preauricular tags	+	+	-	+	-	-	-	+	+	-	+	-	-
Auricular stenosis	-	-	-	-	-	-	-	-	-	-	-	-	n.r.
Hearing impairment	-	+	-	-	-	-	-	-	-	-	-	-	n.r.
Eye anomalies													
Microcornea	-	+	-	+	-	-	+	+	+	+	+	+	-
Strabismus	-	+	-	-	+	-	+	-	-	-	-	-	+
Pale disk	+	-	+	-	-	-	n.r.	n.r.	n.r.	n.r.	n.r.	-	+
Nystagmus	-	+	-	-	-	n.r.	n.r.	+	-	-	-	-	+
Myopia	n.r.	-	-	-	-	-	n.r.	n.r.	-	-	-	-	n.r.

**Suppl. Table 2. Continued**

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13 <sup>1</sup>
Skeletal features													
Bell shaped thorax with spaced nipples	-	-	+	+	+	-	+	+	-	-	-	-	n.r.
Long, thin fingers	+	+	-	-	+	-	+	+	+	+	-	-	+
Coxa valga													+
Pes talus valgus/varus	+	+	-	-	-	-	n.r.	n.r.	-	-	n.r.	-	n.r.
Clitoromegaly	-		-	-	-			-		-		-	-
Constipation	+	+	-	-	+	-	-	-	-	-	-	-	+
Gastroesophageal reflux	-	-	-	-	-	n.r.	-	-	n.r.	-	-	n.r.	n.r.
Thin skin	-	-	-	-	-	-	-	-	-	-	-	-	-

<sup>1</sup>Clinical description of this patient was originally reported by Briscioli et al.<sup>6</sup>

n.r., not recorded.

**Supplementary Table 3.** Primer pairs and annealing temperatures used to amplify the *UBE3B* coding sequence, and sizes of PCR products.

Exon	Primer Sequence (5'→3')		T <sub>ann</sub> (°C)	bp
	Forward	Reverse		
1	CTGCTCTTGAGCCCCTTTC	GCTTGTAGCACCTGGAAGG	59	365
2	CCTTCCAGGTGCTACAAGC	CAGGATGTGAGGGTGACAG	60	300
3	TGTCCTCATCTGTAGGAAGG	ACGTAGCTACCTGGATCATG	59	394
4	GTCCATTCAGGTCCACTGC	ACTAGCAACTCTCGTTGATAG	59	344
5	GAGCAGTTGTAAAGTGAACAC	GGATACTGCGATGCAGTCTG	59	345
6	GGCCTCGGATGTGGGACAC	GGAGAGTGGAGAAGTTGCAC	59	222
7	GCCAGATCATACTCTAGGG	CGAGGTAACTCTACCACTC	60	376
8	CTAGGAATTTGAGCACGTTGG	GTGTCTATCACGGCCACTGG	60	337
9	GTGTTTGCTAGCACATCCTC	CTGATGGCCAGGACACAATC	60	278
10	AGCAGCCTCCGGCATTGAC	GGAAAGACAGTGATTTGCATC	60	358
11	CCAGGTCCTAGCTACAGCTC	ACAGGAGCTGTACTTCAGCC	60	333
12	CTGTCTGGCCTGGCTGTGC	GCTAAGACAACTGTCTGCC	62	341
13	CCCACCTTCTTTTCACGCAC	GACACTGTATTTTGGTCTCTC	60	367
14	TCAACCTCGTTTATTGTCTAG	GGGAATAAATGTGCACCTTC	58	340
15	CAGAGCGAGGGAGATCAAGG	CGTCCTGACAGGTGCTTCAC	62	397
16	GAGTATGGTCATGTTCCCATC	CAACCACCGTTTCCTTGTGC	60	373
17	TCTGCTTGCATCTCATCTAAC	AAGGAGTAACAGGAACAGTTC	58	333
18	GATGCTGGGAGAGCTTCGC	GGAGAGGCAGAGGTCAAGTG	60	369
19	GGCTGACAGCAGCCAGATTC	CAGGCAAAGTACCTAGCACTG	60	376
20	CTGGAAATGCCGATGGCACC	CCTGTAGCAGCTACAGCAAC	60	365
21	CAGCCATGGGTGTTCCAG	GAAGCTTCAAAGGCCAGGTC	62	382
22	TGGTTCCCAAGCATGTGCC	TCAACATAAGCGCAGAGAGC	58	262
23	GGTTCAGAAATGACTAGACTG	GGAGCAGCTGGCTTTCTGG	60	337
24	CGTGCCAGCTGTCTGCATG	GTTACTGCTTGGCCTACCG	60	361
25	GACAGAGCAAACACGTGCTAC	CCCTCCTCACTTGGCTCTG	60	324
26	CAGGGACTGGCCAGATCC	CTGCCTCTGGGAGCTGAAG	62	359