Novel Mutations and Unreported Clinical Features in KBG Syndrome

Emanuela Scarano^a Martina Tassone^a Claudio Graziano^b Dino Gibertoni^c
Federica Tamburrino^a Annamaria Perri^a Maria Gnazzo^d Giulia Severi^b
Francesca Lepri^d Laura Mazzanti^a

^aRare Disease Unit, Department of Pediatrics, ^bUnit of Medical Genetics, Department of Medical and Surgical Sciences, University Alma Mater Studiorum, St. Orsola-Malpighi Hospital, and ^cUnit of Hygiene and Medical Statistics, Department of Biomedical and Neuromotor Sciences, University Alma Mater Studiorum, Bologna, and ^dLaboratory of Medical Genetics, Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy

Keywords

ANKRD11 · 16q24.3 deletion · Joint stiffness · KBG syndrome · Macrodontia

Abstract

KBG syndrome is an autosomal dominant disorder caused by pathogenic variants within *ANKRD11* or deletions of 16q24.3 which include *ANKRD11*. It is characterized by distinctive facial features, developmental delay, short stature, and skeletal anomalies. We report 12 unrelated patients where a clinical diagnosis of KBG was suspected and confirmed by targeted analyses. Nine patients showed a point mutation in *ANKRD11* (none of which were previously reported) and 3 carried a 16q24.3 deletion. All patients presented with typical facial features and macrodontia. Skeletal abnormalities were constant, and the majority of patients showed joint stiffness. Three patients required growth hormone treatment with a significant increase of height velocity. Brain malformations were identified in 8 patients. All patients showed behavioral abnormalities and most had developmental de-

lay. Two patients had hematological abnormalities. We emphasize that genetic analysis of *ANKRD11* can easily reach a detection rate higher than 50% thanks to clinical phenotyping, although it is known that a subset of *ANKRD11*-mutated patients show very mild features and will be more easily identified through the implementation of gene panels or exome sequencing. Joint stiffness was reported previously in few patients, but it seems to be a common feature and can be helpful for the diagnosis. Hematological abnormalities could be present and warrant a specific follow-up.

© 2019 S. Karger AG, Basel

KBG syndrome (OMIM 148050) is a rare autosomal dominant disorder with multiple congenital anomalies and intellectual disability (ID), first described by Hermann et al. [1975], characterized by short stature, macrodontia of the permanent central upper incisors, distinctive craniofacial findings, skeletal anomalies, learning difficulties, and neurobehavioral problems [Hermann et al., 1975; Tekin et al., 2004; Morel Swols et al., 2017]. To date,

almost 200 individuals have been reported in the medical literature, but it is likely that KBG syndrome is underdiagnosed because many of its features, including ID, are often mild, and none of the features are a prerequisite for the diagnosis [Sirmaci et al., 2011; Crippa et al., 2015].

Although diagnostic criteria have been proposed by several authors, there is no consensus on the clinical diagnostic criteria for KBG [Skjei et al., 2007; Ockeloen et al., 2015; Low et al., 2016; Murray et al., 2017]. Eight major criteria were initially defined (macrodonia of the upper central permanent incisors, characteristic facial appearance, hand anomalies, neurological involvement, delayed bone age, costovertebral anomalies, short stature, and the presence of a first-degree relative with KBG syndrome) [Skjei et al., 2007]. Later on, delayed bone age and costovertebral anomalies were suggested to be removed, and macrodonia was not considered a mandatory criterion any longer [Ockeloen et al., 2015; Low et al., 2016; Murray et al., 2017]. Minor features that can further help to establish a clinical diagnosis are otitis media and hearing impairment, seizures, cryptorchidism, feeding problems, palatal insufficiency, and delayed anterior fontanelle closure [Low et al., 2016].

KBG is caused by heterozygous mutations in ankyrin repeat domain-containing protein 11 (*ANKRD11*) [Sirmaci et al., 2011] or deletions of 16q24.3 including *ANKRD11* [Willemssen et al., 2010; Isrie et al., 2012; Sacharow et al., 2012]. Overall, the usefulness of clinical diagnostic criteria was challenged by the identification of *ANKRD11*-mutated individuals in large cohorts of patients with developmental disorders who underwent whole-exome sequencing; these individuals often showed less striking clinical features [Low et al., 2016]. Furthermore, it was recognized that some patients (5 to date) with de novo *ANKRD11* mutations showed a nonclassical Cornelia de Lange syndrome (CdLS) phenotype [Ansari et al., 2014; Parenti et al., 2016], and gene panels for CdLS were recently developed to include *ANKRD11*. A possible explanation of the clinical overlap between KBG and CdLS relies on the functional effect of *ANKRD11*, which was shown to be a chromatin regulator that controls histone acetylation and gene expression during neural development [Gallagher et al., 2015].

Here, we describe the clinical and molecular findings of 12 patients, whose clinical features suggested the diagnosis of KBG syndrome. We compare these patients with previously reported cases to further expand and update the phenotypic and mutational spectrum of this condition. We emphasize the importance of clinical phenotyping and the overlap with other developmental disorders.

Patients and Methods

Twenty-two patients with dysmorphic features, learning disabilities and/or behavioral problems as well as macrodonia were referred to our center in Bologna between 2004 and 2017 with suspected KBG syndrome. They all had at least 2 major criteria or 1 major criterion and 2 minor criteria according to Low et al. [2016]. None of these patients were previously reported.

Analysis of all patients included sequencing of *ANKRD11* and array CGH, since MLPA analysis was not available. Analysis of coding regions and splice sites of the *ANKRD11* gene (16p24.3; NM_013275.5) was performed through high-throughput targeted resequencing and analyzed with MiSeq® platform (Illumina, San Diego, CA) on peripheral blood DNA of the patients. Variants analysis was performed with Illumina VariantStudio Software and Integrative Genome Viewer (IGV). Sanger sequencing confirmed all genetic variants detected in the index cases on re-extracted DNA, using standard protocols, and was used to test parental DNA when available. Genome-wide array analysis was performed in all patients using different array platforms according to local protocols by the referring institutions.

Results

Twenty-two unrelated patients were recruited in this study, 15 males and 7 females. Age at diagnosis ranged from 4 to 21 years. Molecular diagnosis was confirmed in 12 patients and clinical characteristics are summarized in Table 1. Among them, 3 patients were diagnosed after the detection of a 16q24.3 deletion encompassing *ANKRD11* by array CGH, while the clinical suspicion was confirmed by the identification of an *ANKRD11* mutation with targeted sequencing in 9 patients. One patient showed clinical signs of mild CdLS (OMIM 122470), and *ANKRD11* was studied after mutations in CdLS genes (sequencing of *NIPBL*, *SMC3*, *SMC1A*, *HDAC8*, and *RAD21*) were excluded. *ANKRD11* defects resulted de novo in the 10 families where parental DNA was available for analysis (three 16q24.3 deletions and 7 point mutations) and familial in 1 patient (P9: the mutation was transmitted by the mother who presented a milder phenotype); parental samples of P5, who harbored a frameshift mutation, were not available (Tables 2, 3).

Prenatal and Perinatal Period

Few complications occurred in pregnancy, specifically: morphologic anomalies of renal and cerebral systems in 2 patients (P10, P11), decreased fetal movements in 3 (P1, P4, and P6), and IUGR in 1 patient (P5). P5 was born preterm; all the others were born at term (38–42 weeks) – 5 patients with cesarean section – and 5 patients showed respiratory distress. Birth parameters were in the

Table 1. Clinical features

	ANKRD11 mutation									Deletion 16q24.3		
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Gender	M	M	M	M	F	M	M	F	F	M	M	F
Prenatal period and birth												
Pregnancy complications	+	–	–	+	+	+	–	–	–	+	+	–
IUGR	–	–	–	–	+	–	–	–	–	–	–	–
Cesarean section	–	–	+	–	–	–	+	+	+	+	–	–
Growth												
Short stature	+	–	+	+	+	+	+	+	+	–	+	–
Craniofacial dysmorphism												
Microcephaly	–	+	–	–	+	–	–	–	+	–	–	–
Brachycephaly	+	–	–	–	+	+	+	–	–	–	+	–
Coarse face	+	+	+	+	+	+	+	+	+	+	+	+
Nose abnormalities	+	+	+	+	+	+	+	+	+	+	+	+
Long philtrum	+	+	+	+	+	+	+	+	+	+	+	+
Oral abnormalities	+	+	+	+	+	+	+	+	+	+	+	+
Macrodonia	+	+	+	+	+	DEC ^a	+	+	+	DEC ^a	+	DEC ^a
Large protruding ears	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal anomalies												
Wide anterior fontanelle	–	–	–	–	–	–	–	–	–	–	–	+
Delayed bone age	+	+	+	+	–	+	+	+	–	+	+	+
Costal anomalies	+	+	+	+	+	+	–	+	+	+	+	+
Spinal anomalies (scoliosis/kyphosis)	+	+	+	+	+	–	+	–	+	–	+	–
Joint stiffness	+	+	+	+	+	–	+	+	+	+	+	+
Hand anomalies												
Clinodactyly	+	+	+	+	+	+	+	+	+	+	+	+
Brachydactyly	+	+	+	+	+	+	+	+	+	+	–	–
Neurological involvement												
Developmental delay	+	–	+	+	+	+	–	+	+	+	+	+
Intellectual disability, IQ <80	+	–	+	+	+	+	+	–	+	+	+	+
Behavioral abnormalities	+	+	+	+	+	+	+	+	+	+	+	+
Epilepsy/EEG anomalies	+	–	+	U	U	+	U	U	U	–	–	–
Brain imaging anomalies	+	+	–	+	–	+	+	U	+	+	+	U
Other features												
Heart defects	+	–	+	–	+	–	–	+	–	–	+	–
Hearing loss	+	–	–	+	–	–	–	–	+	–	–	–
Cryptorchidism	–	+	–	–	NA	–	–	NA	NA	+	–	NA
Kidney abnormalities	+	–	+	–	+	–	–	–	–	+	–	–
Hematological anomalies	–	+	–	–	–	–	–	–	–	–	+	–

DEC, deciduous teeth; IUGR, intrauterine growth restriction; IQ, intellectual quotient; NA, not applicable; P, patient; U, unknown; +, present; –, absent. ^a Macrodonia of decidual teeth.

normal range in 11/12 patients. After birth, 1 female patient (P12) had feeding difficulties and recurrent vomiting.

Facial Features

Facial gestalt was characteristic in all cases and suggested the diagnosis in children and adult patients. Coarse face was typical, with a round face within the first 4–5 years of age and a triangular face afterwards. Synophrys,

epicanthus, and ptosis were frequent, and some patients had hypertelorism or telecanthus. The most specific facial features included the shape of the nose and mouth: a bulbous nose with a prominent nasal bridge, a wide nasal base, thick alae nasi, and anteverted nares. The philtrum was long and flat in young children, the mouth showed an exaggerated cupid bow and thin vermillion of the upper lip (Fig. 1).

Table 2. List of *ANKRD11* mutations

Patients	Nucleotide change	Predicted protein change	Nature of mutation	Origin
P1	c.2194C>T	p.Arg733Ter	Nonsense	De novo
P2	c.3221_3222delAA	p.Lys1074Argfs*27	Frameshift	De novo
P3	c.5957_5958delGA	p.Arg1986Ilefs*45	Frameshift	De novo
P4	c.3974delT	p.Phe1325Serfs*20	Frameshift	De novo
P5	c.3222dupA	p.Glu1075ArgfsTer27	Frameshift	Unknown
P6	c.2866G>T	p.Glu956Ter	Nonsense	De novo
P7	c.7534C>T	p.Arg2512Trp	Missense	De novo
P8	c.2650delG	p.Asp884ThrfsTer93	Frameshift	De novo
P9	c.3339G>A	p.Trp1113Ter	Nonsense	Maternal

One patient (P9) showed a more severe phenotype, with microcephaly and limb abnormalities (short distal phalanges of the hands and pes cavus). Mild CdLS was the first clinical hypothesis.

Macrodonia (defined as width of a permanent upper incisors >0.92 cm) was found in all patients with permanent teeth and in 3 young patients with deciduous teeth. Four patients showed oligodontia (P3–P5 and P8), 4 enamel hypoplasia (P1, P2, P7, and P11), and 1 male patient had partial fusion of superior central and lateral incisors (P10).

Skeletal Findings

Skeletal anomalies were present in all patients. One patient had bilateral Perthes disease (P1) and another had congenital hip dysplasia (P9); accessory cervical ribs and/or costal hypoplasia were present in 6 patients (P1–P3, P5, P6, and P10), costal hypoplasia in 3 (P4, P8, and P12), scoliosis in 7 (P1–P3, P4, P7, P9, and P11) and kyphosis in 5 (P1, P2, P3, P5, and P9); sternal abnormalities were found in 5 (P2–P4, P10, and P11). P12 had wide anterior fontanelle with delayed closure. A majority of the patients (11/12) showed joint stiffness. Typical hand anomalies were present in all individuals. Clinodactyly and brachydactyly were also detected in the feet.

Growth and Puberty

Stature was below –1 SD in 9/12 cases of which 6 patients (P1, P3, P5–P7, and P9) had a height below the 3rd centile and 3 between the 3rd and 10th centile (P4, P8, and P11).

P7, P9, and P11 required growth hormone (GH) treatment, and 2 of them showed a GH deficit (GH peak was <8 ng/mL in 2 arginine tests; P9: 5,1 ng/mL and 2,2 ng/mL, respectively, and P11: 3,2 ng/mL and 3,9 ng/mL, respectively). Bone age was retarded in these patients. Dur-

Table 3. Genome coordinates of *ANKRD11* mutations

Patients	Genome coordinates, hg19	Origin
P10	Chr16:89283689_89429735, <i>ANKRD11</i> and <i>ZNF778</i>	De novo
P11	Chr16:89335428_89559189, <i>ANKRD11</i>	De novo
P12	Chr16:89429676_89559189, <i>ANKRD11</i>	De novo

ing GH therapy, patients showed a significant increase of height velocity, and their height increased by 1.0 SD (Fig. 2). Only 2 male patients were adult and reached a final height of 151.6 cm (P1) and 172.1 cm (P2), respectively.

Advanced puberty was reported in 2/7 patients (P1, P3), both males, evaluated in peripubertal age. Triptorelin therapy was used in 1 case (P1) and interrupted after 2 months due to a local adverse effect (skin abscesses).

Associated Malformations

Heart defects were reported in 5 patients. P11 had a ventricular septal defect, aortic insufficiency, and a patent foramen ovale; P3 had a ventricular septal defect and patent ductus arteriosus, and P1, P5, and P8 had mild mitral insufficiency, which was associated with tricuspid insufficiency in P5.

Hearing loss was present in 3 patients (P1, P4, and P9), and in 2 cases it was associated with ossicular malformations (P1, P4).

P2 and P10 (2/8 male patients) required surgery for cryptorchidism. Renal abnormalities were present in 4 patients: P3 and P10 showed duplication of renal pelvis, P1 developed nephrotic syndrome, and vesicoureteral reflux in P5 was surgically treated.

Ocular problems were reported in 6/12, in particular astigmatism and strabismus.

Fig. 1. Photographs of patients with *ANKRD11* mutations (P1, P2, P5–P9) or 16q24.3 deletion (P11). Note the typical round or triangular face, synophrys, prominent nose, and long philtrum.

Fig. 2. Growth curves in P7 (a), P9 (b), and P11 (c) treated with growth hormones. Red arrow: beginning of growth hormone therapy. Blue arrow: target height.

Table 4. Summary of clinical features in KBG patients in our study and the literature

	Our study			Literature			Total		
	total	mutation	deletion	total	mutation	deletion	total	mutation	deletion
Cases	12 (100)	9 (75.0)	3 (25.0)	183 (100)	130 (71.0)	53 (29.0)	195 (100)	139 (71.3)	56 (28.7)
Gender									
Male	8/12 (66.7)	6/9 (66.7)	2/3 (66.7)	101/166 (61.0)	72/121 (59.5)	29/45 (64.4)	109/178 (61.2)	78/130 (60.0)	31/48 (65.0)
Female	4/12 (33.3)	3/9 (33.3)	1/3 (33.3)	65/166 (39.0)	49/121 (40.5)	16/45 (35.6)	69/178 (38.8)	52/128 (40.0)	17/48 (35.0)
Unknown	–	–	–	17/183 (9.3)	9/130 (6.9)	8/53 (15.1)	17/195 (8.7)	9/139 (6.5)	8/56 (14.3)
Short stature	9/12 (75.0)	8/9 (88.9)	1/3 (33.3)	102/177 (57.6)	79/127 (62.2)	23/50 (46.0)	111/189 (58.7)	87/136 (64.0)	24/53 (45.3)
Craniofacial dysmorphism	12/12 (100)	9/9 (100)	3/3 (100)	168/170 (98.8)	116/117 (99.1)	52/53 (98.1)	180/182 (98.9)	125/126 (99.2)	55/56 (98.2)
Typical facial gestalt	12/12 (100)	9/9 (100)	3/3 (100)	156/181 (86.2)	105/128 (82.0)	51/53 (96.2)	168/193 (87.0)	114/137 (83.2)	54/56 (96.4)
Long protuberant philtrum	12/12 (100)	9/9 (100)	3/3 (100)	92/149 (61.7)	69/108 (63.9)	23/41 (56.1)	104/161 (64.6)	78/117 (66.7)	26/44 (59.1)
Nose anomalies	12/12 (100)	9/9 (100)	3/3 (100)	134/165 (81.2)	97/120 (80.8)	37/45 (82.2)	146/177 (82.5)	106/129 (82.2)	40/48 (83.3)
Oral findings	12/12 (100)	9/9 (100)	3/3 (100)	108/154 (70.1)	75/109 (68.8)	33/45 (73.3)	120/166 (72.3)	84/118 (71.2)	36/48 (75.0)
Large and prominent ears	12/12 (100)	9/9 (100)	3/3 (100)	92/127 (72.4)	74/99 (74.7)	18/28 (64.3)	104/139 (74.8)	83/108 (76.9)	21/31 (67.7)
Macrodontia	12/12 (100)	9/9 (100)	3/3 (100)	121/143 (84.6)	95/108 (88.0)	26/35 (74.3)	133/154 (86.3)	104/117 (88.9)	29/37 (78.3)
Skeletal anomalies	12/12 (100)	9/9 (100)	3/3 (100)	79/105 (75.2)	61/77 (79.2)	18/28 (64.3)	91/117 (77.8)	70/86 (81.4)	21/31 (67.7)
Delayed bone age	10/12 (83.3)	7/9 (77.8)	3/3 (100)	43/86 (50.0)	34/63 (54.0)	9/23 (39.1)	54/98 (55.1)	42/72 (58.3)	12/26 (46.2)
Costovertebral anomalies	11/12 (91.6)	8/9 (88.8)	3/3 (100)	22/105 (21.0)	21/79 (26.6)	1/26 (3.8)	33/117 (28.2)	28/88 (31.8)	4/29 (13.7)
Spine anomalies	8/12 (66.7)	7/9 (77.8)	1/3 (33.3)	20/86 (23.3)	13/60 (21.7)	7/26 (26.9)	28/98 (28.6)	20/69 (29.0)	8/29 (27.6)
Joint stiffness	11/12 (91.6)	8/9 (88.8)	3/3 (100)	6/27 (22.2)	3/16 (18.8)	3/11 (27.3)	17/39 (43.5)	11/25 (44.0)	6/14 (42.9)
Hand anomalies	12/12 (100)	9/9 (100)	3/3 (100)	126/166 (75.9)	93/119 (78.2)	33/47 (70.2)	138/178 (77.5)	102/128 (79.7)	36/50 (72.0)
Neurological involvement	12/12 (100)	9/9 (100)	3/3 (100)	175/180 (97.2)	124/128 (96.9)	51/52 (98.1)	187/192 (97.4)	133/137 (97.1)	54/55 (98.2)
Developmental delay	10/12 (83.3)	7/9 (77.8)	3/3 (100)	140/178 (78.7)	96/129 (74.4)	44/49 (89.8)	150/190 (78.9)	103/138 (74.6)	47/52 (90.4)
Intellectual disability	10/12 (83.3)	7/9 (77.8)	3/3 (100)	157/173 (90.7)	113/124 (91.1)	44/49 (89.8)	167/185 (90.3)	120/133 (90.2)	47/52 (90.4)
Behavioral features	12/12 (100)	9/9 (100)	3/3 (100)	104/168 (61.9)	81/119 (68.1)	23/49 (46.9)	116/180 (64.4)	90/128 (70.3)	26/52 (50.0)
Epilepsy/convulsion	3/7 (42.8)	3/4 (75.0)	0/3 (0)	52/173 (30.1)	39/124 (31.5)	13/49 (26.5)	55/180 (30.5)	42/128 (32.8)	13/52 (25.0)
EEG anomalies	3/7 (42.8)	3/4 (75.0)	0/3 (0)	61/122 (50.0)	45/81 (55.5)	15/40 (37.5)	64/128 (50.0)	48/85 (56.5)	15/43 (34.9)
Brain imaging anomalies	8/10 (80.0)	6/8 (75.0)	2/2 (100)	36/84 (42.9)	19/56 (33.9)	17/28 (60.7)	44/94 (46.8)	25/64 (39.0)	19/30 (63.3)

Values represent number of cases with percentages in parentheses.

Skin abnormalities were found in several patients, such as hyperpigmented skin, mild ichthyosis, and dyschromic skin.

Hematological anomalies were reported in 2/12 patients: leukopenia and thrombocytopenia (P2) as well as normochromic anemia (P11). P2 showed hypoplasia of bone marrow cells, but specific analysis of peripheral blood (FISH for chromosome 7 monosomy, deletion 5q syndrome, chromosome 8 trisomy, 20q deletion, and *TERC* sequencing) were negative.

Two patients had subclinical hypothyroidism (P1, P7) and another (P9) showed a small thyroid for age. Intense headaches and vertigos were reported in P1 and P9.

Neurological Abnormalities and Behavioral Issues

Brain imaging was performed in 10 patients, and 8 of them showed unspecific structural abnormalities: enlarged cisterna magna (P1, P6, P9, and P10), arachnoid cysts (P2, P4), leptomeningeal cyst (P9), Dandy Walker complex (P1), and Chiari malformation (P6).

Developmental delay and ID (ranging from mild to severe) were reported in 83.3% of the patients, and speech

delay was the most common concern. All the patients showed abnormal behavior. Most patients had nonspecific behavioral traits such as hyperactivity, attention deficit, anxiety, lack of self-confidence, frustration intolerance, aggressiveness, and depression. P2 received a diagnosis of attention deficit/hyperactivity disorder. Sleep disorders occurred in P3, P6, and P11.

Patients without *ANKRD11* Mutations

Ten patients who were selected for *ANKRD11* analysis turned out negative, and array CGH did not identify deletions of the 16q24.3 region. They all had macrodontia and features that would satisfy the clinical criteria for KBG syndrome, although, retrospectively, facial gestalt was less typical. In one of these patients, array CGH identified a large (17.75 Mb) duplication of 12.q21.1q21.33 that was confirmed by karyotype analysis and turned out to be de novo. The patient was a 12-year-old boy with normal growth and border-line intellectual functioning, a speech delay, EEG abnormalities, costal anomalies, facial features including sinophrys, bulbous tip of the nose, thick lips, macrodontia, and 3 supernumerary teeth.

After clinical reevaluation of another patient, mild signs suggestive for CdLS were noted, and a causative mutation of *RAD21* was identified. The remaining 8 patients do not have a specific molecular diagnosis yet.

Discussion

We describe 12 patients who were selected, based on clinical features, for targeted analysis of *ANKRD11*: 9 were found to carry a heterozygous mutation within *ANKRD11*, and 3 had a 16q24.3 deletion identified by array CGH (Tables 2, 3). A summary of the major clinical features in our cohort of patients and in KBG syndrome patients previously reported in literature is shown in Table 4. All 12 patients showed clinical features suggestive of this condition, especially distinctive craniofacial features (Fig. 1).

Overall, these individuals do not differ significantly from previous cohorts, but some inconsistencies are worth mentioning. Short stature (<10th centile in 9/12 patients) and skeletal abnormalities (12/12) are more frequent than previously reported. A contribution to short stature may come from advanced puberty, which was reported in 2/7 male patients in peripubertal age. We believe that early signs of puberty must be carefully monitored in patients diagnosed at a young age. Furthermore, possible benefits of GH therapy have already been reported [Reynaert et al., 2015] and should be evaluated. Costovertebral anomalies were initially considered as a major criterion according to Skjei et al. [2007], but later it was proposed to exclude them from the clinical criteria needed for KBG diagnosis. All our patients had costovertebral anomalies (accessory cervical ribs, costal hypoplasia, scoliosis, and/or kyphosis), and they were helpful for the diagnosis. Joint stiffness was present in a large proportion of our patients, and we point out that this is a frequent but underestimated sign.

Considering that the diagnosis of KBG syndrome in all of these patients was suspected clinically, it is not surprising that specific clinical features are more common with respect to cohorts of patients with unknown diagnosis, who had been referred for ID or congenital defects, where *ANKRD11* mutations were identified by exome sequencing (or chromosomal microarray analysis). Furthermore, all these patients were first referred to a center with a specific expertise on rare disorders and genetics of short stature, where patients with ID and skeletal anomalies will be referred more frequently than patients with isolated ID.

As expected, patients showed a variable set of neurodevelopmental issues [Lo-Castro et al., 2013]. ID ranged from mild to severe and was present in 10/12 patients. Although rather unspecific, every patient had behavioral anomalies, with a single case of diagnosed attention deficit/hyperactivity disorder. No patients had a recognized diagnosis of autism spectrum disorder. Reports of this disorder in KBG are heterogeneous; it was reported to be as high as 47% by some authors [Ockeloen et al., 2015], but much lower by others [Murray et al., 2017]. This difference may be due to varying ascertainment practices and referrals in different countries; such variability has already been noted for other conditions [Schneider et al., 2014]. Seizures and EEG anomalies were present in 25% of patients. Ten patients underwent brain imaging, and cerebral abnormalities were found to be frequent (8/10) but unspecific: 4 patients showed enlarged cisterna magna, 2 had a pineal cyst, 1 had a leptomeningeal cyst, 1 Dandy-Walker malformation, and 1 Chiari malformation.

Regarding long-term follow-up of neurodevelopmental disorders in individuals with KBG, only 2 were adults, but both led an autonomous life. Furthermore, the mother of patient P9 was recognized retrospectively to be affected but had a job and had no problems in taking care of her children. Thus, the neurological outcome can be relatively benign, and we should stress that an early diagnosis can enable an intervention on behavioral issues and help to support adult life and independent living.

Two patients showed hematological abnormalities (one had a pancytopenia with bone marrow hypoplasia and the second had anemia and leukopenia). Only 1 patient was previously reported with acute myeloid leukemia [Goldenberg et al., 2016], and a role of *ANKRD11* in cancer predisposition was proposed, but not confirmed [Nielsen et al., 2008; Lim et al., 2012]. The presence of thrombocytopenia was pointed out as a possible feature distinguishing between KBG syndrome and 16q24 microdeletion syndrome, where *ANKRD11* flanking genes are also involved [Novara et al., 2017]. *ZFPM1* haploinsufficiency was highlighted as the possible cause of thrombocytopenia in individuals with deletions, but we show that a hematological disorder can be present in patients with *ANKRD11* point mutations, although it is not a frequent feature.

We confirm that most *ANKRD11* mutations are loss of function, without mutational hot spots. We report 1 single patient with a missense mutation (P7; p.Arg2512Trp); missense variants are uncommon among KBG patients, and the definition of pathogenicity is difficult. P7 has a typical KBG phenotype, and the p.Arg2512Trp vari-

ant arose de novo affecting a conserved amino acid. Furthermore, a previously reported patient has a missense variant affecting the same amino acid position (p.Arg2512Gln) [Walz et al., 2015]. Thus, we believe that this variant is pathogenic and that an Arginine in position 2512 is likely fundamental for ANKRD11 function. Of the three 16q24.3 deletions we identified, 2 are intragenic and 1 extends to *ZNF778*; P10 has typical clinical features, and *ZNF778* haploinsufficiency is likely irrelevant.

Among 10 patients with a clinical suspicion of KBG syndrome but without an *ANKRD11* defect, an alternative molecular diagnosis was reached in 2 of them. One has a large de novo 12q21.1q21.33 duplication, identified by array CGH and confirmed by standard karyotyping. Interstitial 12q duplications are very rare and can have little or no phenotypic effect: Barber et al. [2007] described a large transmitted duplication of 12q with partial overlap to the one we describe here that had no apparent clinical consequences. Truly, this patient is mildly affected and has no overt ID. It will be interesting to collect further patients with 12q duplications to prove whether clinical overlap with KBG syndrome is actually present. Another patient was tested, after clinical reevaluation, with a gene panel for CdLS and was found to carry a *RAD21* mutation. The remaining 8 patients are good candidates for whole-exome sequencing, although a clinical reevaluation is currently ongoing: we cannot exclude that patients with a typical phenotype carry an undetected *ANKRD11* mutation, e.g., an intragenic rearrangement, since MLPA analysis was not performed.

Clinical features of KBG syndrome can be unspecific and other disorders should be considered in the differential diagnosis. A clinical overlap with Silver-Russell syndrome (OMIM 180860) has been observed [Spengler et al., 2013], but none of our patients had clinical features suggestive of this condition. The overlap with mild CdLS is well established and confirmed here, since 1 patient was found to carry an *ANKRD11* mutation after the exclusion of pathogenic variants in CdLS genes, and a second patient was tested for CdLS genes and had a pathogenic variant in *RAD21*, after *ANKRD11* mutations were excluded. We certainly favor the development of CdLS gene panels that include *ANKRD11*.

CdLS is included in the group of cohesinopathies, human diseases due to germinal mutations in the cohesin genes. Cohesin is a multiprotein complex first identified for its role in proper chromosome segregation and is an essential regulator of most aspects of chromosome biology, including regulation of gene expression, DNA repair, chromatin structure, and genome organization

[Watrin et al., 2016]. ANKRD11 represses the transcriptional activation of target genes of nuclear receptors by recruiting deacetylases to different promoters [Zhang et al., 2004]. Both ANKRD11 and cohesin-complex are involved in gene expression regulation, and dysregulation of functionally interconnected sets of genes due to deficiency of the cohesin complex or ANKRD11 may result in overlapping phenotypic features [Parenti et al., 2016].

Finally, it is interesting to note that at least 5 patients carried a pathogenic *ANKRD11* variant in the context of a dual molecular diagnosis. Four individuals were identified through exome sequencing [Tunovic et al., 2014; Posey et al., 2017] and one through targeted sequencing, in a patient who additionally had a de novo deletion of chromosome 9q [Xu et al., 2013]. This last patient nicely exemplifies the need of detailed phenotype analysis because clinical features were felt to be more complex than expected for KBG syndrome, and array CGH identified a rare interstitial deletion described in patients with ID, short stature, and skeletal anomalies [Mucciolo et al., 2014]. In general, the presence of an independent molecular defect poses a significant hurdle to a correct clinical definition.

Conclusion

KBG syndrome is an emerging condition with a characteristic clinical phenotype in the majority of individuals. The clinical suspicion of KBG should prompt molecular analysis, and an early diagnosis is critical, because patients can benefit from interventions for behavioral issues, which are frequent, and from consideration of GH therapy for short stature. Patients should undergo routine monitoring of hearing, vision, and pubertal status. Based on our findings, hematological abnormalities deserve clinical awareness, and regular blood tests should be considered. Overall, the prognosis is fairly good with appropriate management, ID is often mild, and 2 adult patients that are in follow-up at our Institution lead an autonomous life. In the near future, it is likely that most of the *ANKRD11* mutations will be identified through unbiased strategies, either by comprehensive gene panels or whole-exome sequencing. However, detailed phenotype analysis will be of major help to guide the clinical interpretation of genetic variants.

Acknowledgments

We thank the patients and families for their helpful participation in this work and all the colleagues who have contributed to the data presented here.

Statement of Ethics

Informed consent for genetic analysis and publication of clinical reports and photographs were obtained from the patients or their parents/guardians in compliance with the national ethics regulation.

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

- Ansari M, Poke G, Ferry Q, Williamson K, Aldridge R, et al: Genetic heterogeneity in Cornelia de Lange syndrome (CdLS) and CdLS-like phenotypes with observed and predicted levels of mosaicism. *J Med Genet* 51:659–668 (2014).
- Barber JCK, Maloney VK, Kirchhoff M, Thomas NS, Boyle TA, Castle B: Transmitted duplication of 12q21.32–12q22 includes 48 genes and has no apparent phenotypic consequences. *Am J Med Genet A* 143A:615–618 (2007).
- Cacciari E, Milani S, Balsamo A, Spada E, Bona G, et al: Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). *J Endocrinol Invest* 29:581–593 (2006).
- Crippa M, Rusconi D, Castronovo C, Bestetti I, Russo S, et al: Familial intragenic duplication of *ANKRD11* underlying three patients of KBG syndrome. *Mol Cytogenet* 8:20 (2015).
- Gallagher D, Voronova A, Zander MA, Cancino GI, Bramall A, et al: Ankrd11 is a chromatin regulator involved in autism that is essential for neural development. *Dev Cell* 32:31–42 (2015).
- Goldenberg A, Riccardi F, Tessier A, Pfund R, Busa T, et al: Clinical and molecular findings in 39 patients with KBG syndrome caused by deletion or mutation of *ANKRD11*. *Am J Med Genet A* 170:2847–2859 (2016).
- Herrmann J, Pallister PD, Tiddy W, Opitz JM: The KBG syndrome—a syndrome of short stature, characteristic facies, mental retardation, macrodontia and skeletal anomalies. *Birth Defects Orig Art Ser* 11:7–18 (1975).
- Isrie M, Hendriks Y, Glielissen N, Sistermans EA, Willemsen MH, et al: Haploinsufficiency of *ANKRD11* causes mild cognitive impairment, short stature and minor dysmorphisms. *Eur J Hum Genet* 20:131–133 (2012).
- Lim SP, Wong NC, Suetani RJ, Ho K, Ng JL, et al: Specific-site methylation of tumour suppressor *ANKRD11* in breast cancer. *Eur J Cancer* 48:3300–3309 (2012).
- Lo-Castro A, Brancati F, Digilio MC, Garaci FG, Bollero P, et al: Neurobehavioral phenotype observed in KBG syndrome caused by *ANKRD11* mutations. *Am J Med Genet B Neuropsychiatr Genet* 162B:17–23 (2013).
- Low K, Ashraf T, Canham N, Clayton-Smith J, Deshpande C, et al: Clinical and genetic aspects of KBG syndrome. *Am J Med Genet A* 170:2835–2846 (2016).
- Morel Swols D, Foster J 2nd, Tekin M: KBG syndrome. *Orphanet J Rare Dis* 12:183 (2017).
- Mucciolo M, Magini P, Marozza A, Mongelli P, Mencarelli MA, et al: 9q31.1q31.3 deletion in two patients with similar clinical features: a newly recognized microdeletion syndrome? *Am J Med Genet A* 164A:685–690 (2014).
- Murray N, Burgess B, Hay R, Colley A, Rajagopalan S, et al: KBG syndrome: an Australian experience. *Am J Med Genet A* 173A:1866–1877 (2017).
- Neilsen PM, Cheney KM, Li CW, Chen JD, Cawse JE, et al: Identification of *ANKRD11* as a p53 coactivator. *J Cell Sci* 121:3541–3552 (2008).
- Novara F, Rinaldi B, Sisodiya SM, Coppola A, Giglio S, et al: Haploinsufficiency for *ANKRD11*-flanking genes makes the difference between KBG and 16q24.3 microdeletion syndromes: 12 new cases. *Eur J Hum Genet* 25:694–701 (2017).
- Ockeloen CW, Willemsen MH, de Munnik S, van Bon BW, de Leeuw N, et al: Further delineation of the KBG syndrome phenotype caused by *ANKRD11* aberration. *Eur J Hum Genet* 23:1176–1185 (2015).
- Parenti I, Gervasini C, Pozojevic J, Graul-Neumann L, Azzollini J, et al: Broadening of cohesinopathies: exome sequencing identifies mutations in *ANKRD11* in two patients with Cornelia de Lange-overlapping phenotype. *Clin Genet* 89:74–81 (2016).
- Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, et al: Resolution of disease phenotypes resulting from multilocus genomic variation. *N Engl J Med* 376:21–31 (2017).
- Reynaert N, Ockeloen CW, Sävendahl L, Beckers D, Devriendt K, et al: Short stature in KBG syndrome: first responses to growth hormone treatment. *Horm Res Paediatr* 83:361–364 (2015).
- Sacharow S, Li D, Fan YS, Tekin M: Familial 16q24.3 microdeletion involving *ANKRD11* causes a KBG-like syndrome. *Am J Med Genet A* 158A:547–552 (2012).
- Schneider M, Debbané M, Bassett AS, Chow EW, Fung WL, et al: Psychiatric disorders from childhood to adulthood in 22q11.2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry* 171:627–639 (2014).
- Sirmaci A, Spiliopoulos M, Brancati F, Powell E, Duman D, et al: Mutations in *ANKRD11* cause KBG syndrome, characterized by intellectual disability, skeletal malformations, and macrodontia. *Am J Hum Genet* 89:289–294 (2011).
- Skjei KL, Martin MM, Slavotinek AM: KBG syndrome: report of twins, neurological characteristics, and delineation of diagnostic criteria. *Am J Med Genet A* 143A:292–300 (2007).
- Spengler S, Oehl-Jaschkwitz B, Begemann M, Hennes P, Zerres K, Eggermann T: Haploinsufficiency of *ANKRD11* (16q24.3) is not obligatorily associated with cognitive impairment but shows a clinical overlap with Silver-Russell syndrome. *Mol Syndromol* 4:246–249 (2013).
- Tekin M, Kavaz A, Berberoğlu M, Fitoz S, Ekim M, et al: The KBG syndrome: confirmation of autosomal dominant inheritance and further delineation of the phenotype. *Am J Med Genet A* 130A:284–287 (2004).
- Tunovic S, Barkovich J, Sherr EH, Slavotinek AM: De novo *ANKRD11* and *KDM1A* gene mutations in a male with features of KBG syndrome and Kabuki syndrome. *Am J Med Genet A* 164A:1744–1749 (2014).
- Walz K, Cohen D, Neilsen PM, Foster J 2nd, Brancati F, et al: Characterization of *ANKRD11* mutations in humans and mice related to KBG syndrome. *Hum Genet* 134:181–190 (2015).
- Watrin E, Kaiser FJ, Wendt KS: Gene regulation and chromatin organization: relevance of cohesin mutations to human disease. *Curr Opin Genet Dev* 37:59–66 (2016).
- Willemsen MH, Fernandez BA, Bacino CA, Gerkes E, de Brouwer AP, et al: Identification of *ANKRD11* and *ZNF778* as candidate genes for autism and variable cognitive impairment in the novel 16q24.3 microdeletion syndrome. *Eur J Hum Genet* 18:429–435 (2010).
- Xu M, Zhou H, Yong J, Cong P, Li C, et al: A Chinese patient with KBG syndrome and a 9q31.2–33.1 microdeletion. *Eur J Med Genet* 56:245–250 (2013).
- Zhang A, Yeung PL, Li CW, Tsai SC, Dinh GK, et al: Identification of a novel family of ankirin repeats containing cofactors for p160 nuclear receptors coactivators. *J Biol Chem* 279:33799–33805 (2004).