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ANKRD11 variants: KBG syndrome and beyond

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Abstract

Mutations affecting the transcriptional regulator Ankyrin Repeat Domain 11 (ANKRD11) are mainly associated with the multisystem developmental disorder known as KBG syndrome, but have also been identified in individuals with Cornelia de Lange syndrome (CdLS) and other developmental disorders caused by variants affecting different chromatin regulators. The extensive functional overlap of these proteins results in shared phenotypical features, which complicate the assessment of the clinical diagnosis. Additionally, re-evaluation of individuals at a later age occasionally reveals that the initial phenotype has evolved toward clinical features more reminiscent of a developmental disorder different from the one that was initially diagnosed. For this reason, variants in *ANKRD11* can be ascribed to a broader class of disorders that fall within the category of the so-called chromatinopathies. In this work, we report on the clinical characterization of 23 individuals with variants in *ANKRD11*. The subjects present primarily with developmental delay, intellectual disability and dysmorphic features, and all but two received an initial clinical diagnosis of either KBG syndrome or CdLS. The number and the severity of the clinical signs are overlapping but variable and result in a broad spectrum of phenotypes, which could be partially accounted for by the presence of additional molecular diagnoses and distinct pathogenic mechanisms.

KEYWORDS
ANKRD11, chromatinopathies, Cornelia de Lange syndrome (CdLS), developmental disorders, KBG syndrome (KBGS)

1 | INTRODUCTION

Transcriptional regulators are key players in numerous biological processes. Ankyrin Repeat Domain 11 (ANKRD11) is an important co-regulator able to induce changes in gene expression by recruiting chromatin remodelers to target genes upon interaction with specific transcriptional repressors or activators.[1,2] The corresponding gene (OMIM *611192) is located at 16q24.3 and encodes a 298 kDa protein of 2663 amino acids containing five ankyrin repeats (amino acids 162–284), two repression domains (amino acids 318–611 and 2369–2663) and one activation domain (amino acids 1851–2145).[1] Due to its unique structure, ANKRD11 is believed to mediate both transcriptional activation and repression.[1,3] ANKRD11 is best characterized for its function as a co-regulator in the developing brain, where it plays a critical role for the proliferation of neural progenitors, for the genesis and positioning of newborn neurons,[4] for neuronal plasticity[5] and for dendritic differentiation.[6]

ANKRD11 was first associated with human disease when deletions at 16q24.3 were identified in individuals with autism spectrum disorder (ASD).[7] Two years later, Willemsen and colleagues provided evidence for a novel microdeletion syndrome by describing four patients characterized by ASD, variable levels of intellectual disability and dysmorphic features carrying interstitial deletions at 16q24.3.[8] Subsequent reports of individuals with intellectual disability, facial dysmorphism, and ASD allowed the narrowing of the minimal common region of overlap of this 16q24.3 microdeletion syndrome to *ANKRD11* only, suggesting a role of *ANKRD11* in neurodevelopment.[9,10]

The first point mutations in *ANKRD11* were identified in seven individuals with KBG syndrome (KBGS, OMIM #148050).[5] This is a rare disorder named after the initials of the first three affected individuals and characterized by intellectual disability, global developmental delay, short stature, skeletal anomalies, distinctive facial features, and macrodontia of the upper central incisors.[11] Since the first description by Sirmaci and colleagues,[5] additional individuals with KBGS have

been reported to carry point mutations, duplications or microdeletions involving *ANKRD11*, thus pointing to *ANKRD11* as the main gene responsible for this syndrome.[12–27] Importantly, a marked inter-familial and intra-familial phenotypical variability has been reported in association with KBGS, indicating variable expressivity and penetrance.[11,20] With the falling cost and increasing accessibility of next generation sequencing technologies and microarrays, variants in *ANKRD11* have also been reported in association with neurodevelopmental syndromes other than KBGS. Specifically, an individual with an initial clinical diagnosis of Silver-Russell syndrome was found to harbor a 348 kb microdeletion at 16q24.3 encompassing *ANKRD11* and *SPG7*.[28] Point mutations in *ANKRD11* were also identified in subjects with phenotypes reminiscent of Cornelia de Lange syndrome (CdLS) (OMIM #122470).[29–32] Loss-of-function variants in *ANKRD11* were similarly described in association with Coffin-Siris syndrome (CSS) (OMIM #135900).[23] Importantly, CdLS and CSS clinically overlap to some extent with KBGS. The shared clinical features include a variable degree of developmental delay and intellectual disability, growth retardation, limb anomalies and characteristic facial dysmorphism.[23,29,30] These findings suggest that variants in *ANKRD11* are not necessarily associated with KBGS only, but that they are rather linked to a larger spectrum of neurodevelopmental syndromes. Accordingly, *ANKRD11* has been described as one of the most frequently mutated genes in individuals with severe developmental disorders.[33,34]

In this work we discuss the clinical and molecular findings of 23 individuals with variants in *ANKRD11* and describe a wide spectrum of phenotypes associated with mutations in this gene.

2 | MATERIALS AND METHODS

2.1 | Cohort

Individuals herein described were recruited thanks to a large international cooperation that includes Germany, Italy, Ireland, Colombia, Canada, and the United States.

Procedures including subjects were initially approved by the Ethical Committee of the University of Lübeck (approval number for human studies HL07-158) and the Ethical Committees of the respective institutions. All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individuals included in this study. An additional informed consent was collected for the publication of subjects' photographs.

Individuals were analyzed by means of exome sequencing, gene panels or microarrays at their respective institutions. Referring physicians provided detailed developmental, neurological, and behavioral history of the subjects. Variants were described on the *ANKRD11* NM_013275.6 RefSeq transcript using HGVS recommendations.[35] All variants have been submitted to the ClinVar database and have been assigned the following accession numbers: SCV001478030–SCV001478045.

2.2 | Facial dysmorphology novel analysis

The Facial Dysmorphology Novel Analysis (FDNA Inc., Boston, MA) technology combines facial recognition software with biological knowledge. This technology allows to detect dysmorphic features and recognizable patterns of facial malformations from 2D facial photographs. Face2Gene (FDNA) was used as a tool for computer analysis of subjects' photographs (https://face2gene.com).[36]

3 | RESULTS

3.1 | Individuals

The group of individuals described herein is composed of 11 females and 12 males, with an age range extending from four to 23 years. The median age of the initial clinical diagnosis was 6 years and 5 months, whereas the median age of the last clinical examination was 9 years and 3 months. Phenotypical appearance of the individuals can be found in Figure 1.

Thirteen individuals received a clinical diagnosis of KBGS (Individuals 1, 8, 10, 11, 14, 15, 17, 18, 19, 20, 21, 22, and 23). Three individuals received an initial clinical diagnosis of CdLS or atypical CdLS that was reconfirmed at a later re-evaluation (Individuals 2, 5, and 6), while five individuals were diagnosed as CdLS during early childhood but were reclassified as KBGS after a re-evaluation later in life (Individuals 3, 4, 7, 9, and 12). Two individuals presented with non-specific syndromic intellectual disability and developmental delay (Individuals 13 and 16). A schematic representation of the diagnostic evaluation of the individuals of the present series is provided in the Figure S1.

An additional clinical analysis was carried out with the Face2Gene database for all individuals for whom photographic material was available (all except Individuals 9 and 14) (Table 1). The software assigned a likely diagnosis of KBGS to 17 out of 21 individuals (Individuals 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 16, 17, 18, 19, 21, and 22). CdLS was considered a possible diagnosis with medium-low probability for Individuals 2, 4, 6, 7, 11, and 12. No obvious diagnosis was assigned to Individuals 5, 15, 20, and 23. Additional syndromes with medium-low similarity scores that were contemplated as differential diagnosis in at least five individuals of our cohort include Wiedemann-Steiner syndrome, fetal alcohol syndrome, PMM2-related disorder and Williams-Beuren syndrome.

3.2 | Clinical features

An overview of the clinical features of each individual is listed in Table 1.

Milestones in motor and verbal development were found to be delayed for all individuals but one: sitting independently was achieved at a median age of 12 months and walking independently at 24 months. The median age of pronouncing the first words was

FIGURE 1 Phenotypical appearance of the following individuals: (A) Individual 1, age 13; (B) Individual 2, age 8; (C) Individual 3, age 7.5; (D) Individual 5, age 17; (E) Individual 6, age 4; (F) Individual 8, age 10; (G) Individual 10, age 9; (H) Individual 11, age 3; (I) Mother of Individual 13, age 21; (J) Individual 15, age 8; (K) Individual 16, age 13; (L) Individual 17, age 9; (M) Individual 18, age 9; (N) Individual 19, age 7; (O) Individual 20, age 6; (P) Individual 21, age 4; (Q) Individual 22, age 5; (R) Individual 23, age 14

24 months. Individuals 11, 13, and 21 are currently still non-verbal at an age of 6, 4, and 15 years, respectively.

Intellectual disability and behavioral problems were also detected in the large majority of the individuals (85% and 68% of subjects, respectively). The level of intellectual disability could be assessed in four individuals and appeared moderate in one and mild in three subjects. Behavioral problems ranged from shyness or inability to recognize and respect personal boundaries to aggressiveness, autistic features and attention deficit hyperactivity disorder.

The most frequent phenotypical features found in our cohort comprise a characteristic face wide at the zygoma (70%), low anterior hairline (65%), synophrys (65%), thick eyebrows (70%), long eyelashes (78%), anteverted nostrils (78%), broad nasal tip (70%), thick alae nasi (65%), long philtrum (83%), macrodontia of central incisors (65%), delayed bone age (67%), short fifth finger (61%), and clinodactyly of the fifth finger (70%).

Additional features commonly observed include arched eyebrows (48%), smooth philtrum (48%), thin upper vermilion (52%), brachydactyly (48%), small hands and feet (52%), proximally set thumbs (48%), visual problems (50%), and delayed dentition (50%).

3.3 | Clinical re-evaluation and age-dependent phenotypical evolution

Five individuals of the present cohort were diagnosed as CdLS during early childhood but were reclassified as KBGS after phenotypical re-evaluation (Subjects 3, 4, 7, 9, and 12). Table 2 provides an

TABLE 1 Clinical features of ANKRD11 individuals

Individuals Information	Individual number	1	2	3	4	5	6	7	8	9	10	11	12
	Country	Germany	Germany	Germany	Italy	Germany	Italy	Italy	Germany	Germany	Germany	Germany	Germany
	Gender	f	m	f	f	f	f	f	m	m	m	m	m
	Suspected clinical diagnosis	KBG	CdLS	CdLS/KBG	CdLS/KBG	CdLS	CdLS	CdLS/KBG	KBG	CdLS/KBG	KBG	KBG	CdLS/KBG
	Primary diagnosis Face2Gene	KBG (high)	KBG (high)	KBG (high)	KBG (high)	KBG (low)	KBG (high)	CdLS (medium)/KBG (medium)	KBG (high)	NA	KBG (high)	KBG (high)	KBG (high)
	Secondary diagnosis Face2Gene	Wiedemann-Steiner syndrome (medium)	CdLS (low), fetal alcohol syndrome (low), PMM2-related disorders (low)	CHARGE syndrome (medium), fetal alcohol syndrome (low), Wiedemann-Steiner syndrome (low)	CdLS (medium), fetal alcohol syndrome (medium), Adams-Oliver syndrome (medium)	PMM2-related disorders (low), Phelan-McDermid syndrome (low)	Wiedemann-Steiner syndrome (medium), CdLS (low)	Hyperphosphatasia with mental retardation syndrome (medium), Williams-Beuren syndrome (low)	Williams-Beuren syndrome (medium), Beckwith-Wiedemann syndrome (medium), fetal alcohol syndrome (low)	NA	Williams-Beuren syndrome (medium), PMM2-related disorders (medium), Moebius syndrome (medium)	PMM2-related disorders (medium), CdLS (medium)	CdLS (medium), Aarskog-Scott syndrome (medium), Wiedemann-Steiner syndrome (low)
Mutation	Gene (RefSeq NM_013275.6)	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*
	Exon	9	9	9	9	9	9	9	9	9	9	9	IVS 9
	DNA change	c.915delA	c.1711_1723del	c.1977C > A	c.2398_2401delGAAA	c.2408_2412delAAAAA	c.2692C > T	c.7356dupC	c.7411_7422del	c.1903_1907delAAACA	c.4218C > A	c.4087C > T	c.7470 + 2 T > C
	Protein change	p.(Pro306Hisfs*62)	p.(Thr571Alafs*15)	p.(Tyr659*)	p.(Glu800Asnfs*62)	p.(Lys803Argfs*5)	p.(Arg898*)	p.(Lys2453Glnfs*79)	p.(Thr2471_Gly2474del)	p.(Lys635Glnfs*26)	p.(Tyr1406*)	p.(Arg1363*)	
	rs number				rs797045027 (pathogenic)	rs886039902 (pathogenic)				rs886041125 (pathogenic)		rs1364690005 (pathogenic)	
	Origin	de novo	NA	NA	Inherited	de novo	de novo	de novo	de novo	NA	de novo	de novo	Inherited
Auxological data at birth	Gestational weeks	37 + 4	38	39	36	40	37	36 (twin)	41 + 2	37 + 3	38 + 6	37	26
	Length	51 cm (0.42 SD)	51 cm (−0.04 SD)	48 cm (−1.7 SD)	NA	51 cm (−0.32 SD)	51 cm (0.63 SD)	48 cm (−0.15 SD)	57 cm (1.64 SD)	46 cm (−1.95 SD)	48 cm (−1.65 SD)	48 cm (−0.92 SD)	NA
	Weight	3320 g (0.53 SD)	3160 g (−0.34 SD)	3250 g (−0.54 SD)	1940 g (−1.83 SD)	3840 g (0.87 SD)	2200 g (−1.78 SD)	2500 g (−0.56 SD)	3790 g (0.08 SD)	2420 g (−1.75 SD)	2620 g (−1.94 SD)	1990 g (−2.5 SD)	850 g (−0.18 SD)
	OFC	35 cm (0.64 SD)	36 cm (0.79 SD)	33 cm (−1.77 SD)	NA	NA	NA	31 cm (−1.53 SD)	35.5 cm (−0.34 SD)	33 cm (−1.11 SD)	32.5 cm (−2.09 SD)	30.5 cm (−2.6 SD)	NA
Auxological data at latest evaluation	Age	13 years 2 months	6 years 1 months	7 years 6 months	9 years	17 years	10 years	3 years 4 months	9 years 4 months	10 years 4 months	8 years 6 months	3 years 4 months	11 year 6 months
	Height	152 cm (−1.13 SD)	119.5 cm (0.09 SD)	108 cm (−3.27)	122.5 cm (−2.12 SD)	150 cm (−2.6 SD)	145 cm (0.54 SD)	93.5 cm (−1.32 SD)	135 cm (−0.53)	133.4 cm (−1.39 SD)	137 cm (0.64 SD)	90 cm (−2.36 SD)	141 cm (−1.04 SD)
	Weight	48.5 kg (−0.07 SD)	21 kg (−0.26 SD)	21 kg (−1.14)	23 kg (−1.73 SD)	43 kg (−2.36 SD)	41 kg (0.95 SD)	12 kg (−1.79 SD)	29 kg (−0.53)	35.1 kg (0.04 SD)	28.9 kg (0.03 SD)	12 kg (−1.99 SD)	27 kg (−2.17 SD)
	OFC	54 cm (−0.03 SD)	51.2 cm (−0.76 SD)	50 cm (−1.42 SD)	51.5 cm (−0.63 SD)	52 cm (−2.66 SD)	50 cm (−2.24 SD)	49.1 cm (−0.51 SD)	55 cm (1.22 SD)	NA	53 cm (−0.06 SD)	46.5 cm (−3.51 SD)	53 cm (−0.91 SD)
Head	Brachycephaly	−	−	−	−	−	+	−	−	−	−	+	−
	Microcephaly	−	−	−	−	−	−	−	−	+	−	+	−
	Low anterior hairline	−	+	−	NA	+	+	+	−	+	−	+	+ mild
	Sparse scalp hair	−	−	−	−	−	−	−	−	−	−	−	−
	Flat facies	−	−	−	−	−	+	−	−	+	−	−	+ mild
	Coarse facies	−	−	−	−	−	+	±	−	−	−	−	−
	Round/triangular facies	−	−	+	+	+	+	−	−	+	−	−	+ triangular
	Frontal bossing	−	−	−	−	−	−	−	−	-	−	−	−
Eyes	Arched eyebrows	−	+ mild	+	−	−	−	+	- (straight)	+	−	−	+
	Thick eyebrows	+	−	+	+	+	+	+	- (marked)	+	−	+	+
	Synophrys	+	−	+	+	+	+	+	−	-	−	+	−
	Long eyelashes	+	+ mild	+	−	−	+	+	−	+	+	+	Not long, but thick/ prominent
	Visual problems	−	+ lacrimal duct stenosis (left), strabismus	+ myopia, strabismus	NA	−	+	−	−	-	−	−	+ retinopathy, strabismus
Nose	Depressed nasal bridge	−	−	−	−	−	+	−	−	-	+	+	−
	Anteverted nostrils	−	+	+	+	+	+	+	+	+	+	+	+
	Thick alae nasi	+	−	+	−	+	−	−	−	+	−	+	−
	Broad nasal tip	+	+	+	−	+	+	−	+	+	−	+	+
	Long philtrum	−	+	+	+	+	+	+	+	+	+	−	+
	Smooth philtrum	−	+	- prominent	−	−	−	−	+	+	+	+	+

(Continues)

TABLE 1 (Continued)

Mouth	Large mouth	–	–	–	+	–	+	–	–	+	–	+	–
	Downturned corners of the mouth	–	+	–	–	–	+	–	+	-	–	+	–
	Thin upper vermilion	–	+	+	–	+	+	+	+	–	–	+	–
	Thick lower vermilion	–	+	–	–	–	–	–	+	+	+	–	+ mild
	Macrodontia of central incisors	+	NA	+	+	–	+	–	+	+ and Talon cusps	+	–	+
	Delayed dentition	NA	NA	NA	NA	NA	+	–	NA	–	–	–	NA
Skin	Hypertrichosis	–	–	+	–	+	+	+	–	NA	–	–	+
	Hairy elbows	–	–	–	–	NA	+	–	–	–	–	–	–
Skeletal	Small hands/feet	–	+	+	+	+	–	–	–	+	Small toes, normal lenght of hands and feet	–	–
	Brachydactyly	–	+	+	+	+	–	–	Mildly broad and short terminal phalanges	+	–	–	+ mild
	Proximally set thumbs	–	–	–	+	+	–	+	–	-	+ slightly	–	+
	Clinodactyly fifth finger	–	+ mild	–	+	–	–	–	+ mild	+	+	+	–
	Short fifth finger	+	+ mild	+	+	+	–	–	+ mild	+	+	+	–
	A/Hypoplasia of the distal phalanges of the fifth finger	–	–	–	+	+	–	–	–	-	Short middle phalanx	–	–
	A/Hypoplastic nails	–	–	–	–	+	+	–	–	-	–	–	–
	Syndactyly of toes	–	–	+	NA	–	–	–	–	-	Mild basal syndactyly II/III hand and feet	–	–
	Prominent interphalangeal joints	–	–	–	–	+	–	–	–	-	–	–	–
	Joint laxity	NA	NA	–	NA	–	- (pes planum)	–	NA	-	–	–	–
	Delayed bone age	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	+
Other	Cardiac anomalies	–	+ peripheral pulmonary stenosis (normalized)	–	–	–	–	–	–	-	+ ASD (subclinical) / VSD (spontaneously closed)	–	–
	Genitourinary anomalies	–	–	–	–	–	–	–	–	+ cryptorchidism	–	–	NA
	CNS anomalies	–	–	–	–	–	+	NA	MRI: small cerebellar vermis, arachnoidal cyst	+	no MRI performed	–	- (mildly enlarged outer CNF spaces fronto-temporal)
	Feeding problems	–	+	–	–	–	–	–	–	-	–	+	+ in infancy
	Hearing problems	+	–	–	NA	–	–	–	–	-	–	–	–
	Seizures	–	–	–	–	–	–	–	One seizure, EEG intermittent deceleration right parieto-occipital (no medication)	+	–	–	+ (for 2 years starting at age 4 years 6 months, Valproate until age 10, now seizure-free without medication but EEG changes)
	Frequent infections	–	–	–	–	–	–	–	–	-	–	–	- (but bronchial problems due to prematurity)
Cognitive development	ID	+	- (IQ:104)	+	+	–	+ (IQ:39)	+ (mild)	–	+	+ (IQ-Test 2014: 78; IQ-Test 2016: 58)	+	+ (IQ: 50–75)
	Behavioral problems	+ ASD-like	+	+	+	NA	+	–	–	+	+aggressiveness, trouble in social groups	–	+ ADHD
Motor development	Age of sitting independenty	NA	17 months	NA	NA	NA	12 months	9 months	NA	18 months	13–14 months	3 years	NA
	Age of walking independently	after 18 months	22 months	NA	NA	18 months	15 months	24 months	22 months	24 months	24 months	3.5 years	18–19 months
Verbal development	Age of first words	after 12 months	2 years	Delayed	NA	24 months	18 months	16 months	18 months	4 years	24 months	No speech development	30 months
Other		Hypopigmentation	Epicanthus, upslanting palpebral fissures, large protruding ears, rhinophonia aperta, velopharyngeal incompetence (surgically corrected), muscular hypotonia			Congenital hip dysplasia			Muscular hypotonia, protruding ears		High palate, maxillary prognatism, vermillion of upper lip tended		Pulmunary displasia, cerebral hemorrage

TABLE 1 (Continued)

Individuals Information	Individual number	13	14	15	16	17	18	19	20	21	22	23
	Country	Germany	Colombia	Colombia	Italy	Italy	Italy	Canada	Ireland	USA	USA	USA
	Gender	m	f	f	m	m	m	f	m	f	f	m
	Suspected clinical diagnosis	DD + ID	KBG	KBG	DD + ID	KBG	KBG	KBG	KBG	KBG	KBG	KBG
	Primary diagnosis Face2Gene	KBG (high)	NA	KBG (low)	KBG (high)	KBG (high)	KBG (high)	KBG (high)	Noonan (low)	Hyperphosphatasia-Mental Retardation (medium)	KBG (high)	Neurofibromatosis type 1 (medium)
	Secondary diagnosis Face2Gene	Williams-Beuren syndrome (medium), PMM2-related disorders (low)	NA	Angelman syndrome (low), Coffin-Siris syndrome (low), Prader-Willi syndrome (low)	Floating-Harbor syndrome (medium), Wiedemann-Steiner syndrome (medium), PMM2-related disorders (medium)	Kabuki syndrome (medium), Bardet-Biedl syndrome (medium)	Fetal alcohol syndrome (medium), Noonan syndrome (medium)	Hyperphosphatasia with mental retardation syndrome (low), Moebius syndrome (low)	Kabuki (low), Silver-Russell (low), Williams-Beuren (low), Aarskog-Scott (low), KBG (low)	KBG (medium), Smith-Magenis (medium), Rett (medium)	Wiedemann-Steiner (mediuum)	Noonan syndrom (low), Turner syndrome (low), Sotos syndrome (low)
Mutation	Gene (RefSeq NM_013275.6)	*ANKRD11*	*ANKRD11*	*ANKRD11*	*ANKRD11*	ANKRD11	ANKRD11	ANKRD11	ANKRD11	ANKRD11	ANKRD11	ANKRD11
	Exon	9	9	9	9	9	9	9	9	9	9	4–12
	DNA change	c.1381_1384delGAAA	c.1903_1907delAAACA	c.3888dupC	c.3591_3594del	c.1381_1384delGAAA	c.1903_1907delAAACA	c.1903_1907delAAACA	c.2408_2412delAAAAA	c.5123C > A	c.1381_1384delGAAA	chr16:89,335,426-89,371,803del
	Protein change	p.(Glu461Glnfs*48)	p.(Lys635Glnfs*26)	p.(Asn1297Glnfs*3)	p.(Lys1198Argfs*119)	p.(Glu461Glnfs*48)	p.(Lys635Glnfs*26)	p.(Lys635Glnfs*26)	p.(Lys803Argfs*5)	p.(Ser1708*)	p.(Glu461Glnfs*48)	
	rs number		rs886041125 (pathogenic)						rs886039902 (pathogenic)			
	Origin	Inherited	de novo	de novo	de novo	NA	de novo	de novo	de novo	de novo	NA	de novo
Auxological data at birth	Gestational weeks	34 + 3	32	40	31	39	36	40	36	40	NA	NA
	Length	43 cm (−1.39 SD)	49 cm (1.94 SD)	49 cm (−1.23 SD)	35 cm (−2.23 SD)	60 cm (3.45 SD)	50 cm (0.22 SD)	51 cm (0.09 SD)	NA	NA	NA	NA
	Weight	1750 g (−1.65 SD)	2300 g (1.38 SD)	2900 g (−1.36 SD)	990 g (−1.98 SD)	4710 g (2.72 SD)	2600 gr (−0.74 SD)	3870 g (0.86 SD)	2950 g (0.31 SD)	2410 g (−2.26 SD)	NA	NA
	OFC	30 cm (−1.57 SD)	NA	NA	26.2 cm (−1.87 SD)	NA	34 cm (0.01 SD)	34 cm (−0.18 SD)	NA	NA	NA	NA
Auxological data at latest evaluation	Age	1 years 9 months	11 years 11 months	9 years 5 months	13 years	9 years 9 months	7 years	12 years	8 years	15 years 2 months	4 years 3 months	13 years 7 months
	Height	79 cm (−2.21 SD)	139 cm (−1.89 SD)	131 cm (−1.12 SD)	157.4 cm (−0.48 SD)	147 cm (0.95 SD)	130 cm (0.77 SD)	137 cm (−1.93 SD)	122 cm (−1.01 SD)	146.5 cm (−1.99 SD) at 13 years 8 months	92.2 cm (−2.4 SD)	158 cm (−0.37 SD)
	Weight	11.4 kg (−0.51 SD)	26.4 kg (−2.64 SD)	36.9 kg (0.75 SD)	45.8 kg (−0.35 SD)	67.3 kg (2.83 SD)	23.6 kg (−0.33 SD)	32 kg (−1.47 SD)	22.3 kg (−1.01 SD)	45 kg (−0.41 SD) at 13 years 8 months	12.6 kg (−2.3 SD)	40.9 kg (−1.0 SD)
	OFC	50.8 cm (1.29 SD)	52 cm (−1.19 SD)	49 cm (−2.97 SD)	50 cm (−3.54 SD)	55.7 cm (1.60 SD)	51 cm (−1.28 SD)	52.1 cm (−1.19 SD)	50.5 cm (−1.66 SD)	52.5 cm (−1.03 SD) at 13 years 8 months	46 cm (−3.0 SD)	54.9 cm (−0.19 SD)
Head	Brachycephaly	−	−	−	−	−	−	−	−	−	+	−
	Microcephaly	−	−	+	+	−	+	−	−	−	+	−
	Low anterior hairline	+	−	−	+	+	+	+	+	+	+	−
	Sparse scalp hair	−	+	+	−	−	−	+	−	−	−	−
	Flat facies	+	+	−	+	−	−	−	+	−	+	−
	Coarse facies	−	−	−	−	−	−	−	−	−	−	−
	Round/triangular facies	−	+	+	Ovalar	ovalar	+	+/ wide at zygoma/ diamond	+	+	+ wide at zygoma	+
	Frontal bossing	−	+	+	−	−	−	−	−	−	−	−

(Continues)

TABLE 1 (Continued)

Eyes	Arched eyebrows	+	–	+	–	+	–	–	+	–	+	+
	Thick eyebrows	+	–	–	+	+	+	–	–	+	+	+
	Synophrys	+	+	–	–	+	–	+	+	+	+	+
	Long eyelashes	–	+	+	+	+	+	+	+	+	+	+
	Visual problems	+ iris coloboma	–	+ myopia	–	+ strabismus	+	–	+ hypermetropia and astigmatism	+ delayed visual maturation, exotropia and cortical visual impairment	+ farsighted	+ nystagmus
Nose	Depressed nasal bridge	+	–	–	–	–	–	–	+	–	+	–
	Anteverted nostrils	+	–	+	–	+	–	+	+	+	+	–
	Thick alae nasi	+	+	–	+	+	+	+	+	+	+	+
	Broad nasal tip	+	+	+	+	–	+	+	–	–	–	+
	Long philtrum	–	+	+	–	+	+	+	+	+	+	+
	Smooth philtrum	–	+	+	–	–	+	+	+	–	–	–
Mouth	Large mouth	–	–	+	–	–	–	–	–	+	+	–
	Downturned corners of the mouth	–	+	+	–	–	–	–	–	+	–	–
	Thin upper vermilion	–	+	–	–	–	+	+	+	+	–	–
	Thick lower vermilion	+	–	+	–	–	–	–	+	–	–	–
	Macrodontia of central incisors	NA	–	+	–	+	+	+ fused lateral incisors	–	+	+	+
	Delayed dentition	NA	+	–	+	–	+	+ missing #45, 35, 12 and 22	+ missing teeth	+	- crowded	NA
Skin	Hypertrichosis	–	–	–	–	–	–	–	–	–	–	–
	Hairy elbows	–	+	–	–	–	–	–	–	–	NA	–
Skeletal	Small hands/feet	+	–	–	+	+	–	+	+	+ (foot length 17.8 cm [<third %]at 8 years)	+	–
	Brachydactyly	–	–	+	+	+	–	–	–	-	+	–
	Proximally set thumbs	–	+ slightly	+ slightly	+	–	–	+	+	-	+	+
	Clinodactyly fifth finger	+	+	+	+	–	+	+	+	+ bilaterally	+	+
	Short fifth finger	–	+	+	+	+	–	–	–	-	+	+
	A/Hypoplasia of the distal phalanges of the fifth finger	–	Short middle phalanx	–	–	–	–	+	–	–	+	–
	A/Hypoplastic nails	–	–	–	–	+	–	+	+ second	-	+	+ smaller second nail
	Syndactyly of toes	–	–	–	–	–	+	–	+	-	–	+
	Prominent interphalangeal joints	–	–	–	+	+	–	–	–	-	+	+
	Joint laxity	–	+	+	–	–	–	–	NA	-	–	–
	Delayed bone age	NA	NA	- (precocious puberty)	+	NA	NA	+	–	NA	NA	NA

TABLE 1 (Continued)

Other	Cardiac anomalies	−	−	−	−	−	−	−	−	−	+	+
	Genitourinary anomalies	+ cryptorchidism	−	−	+ cryptorchidism	−	−	+ family history polycystic kidney disease	−	−	−	−
	CNS anomalies	+ ventriculomegaly	no MRI performed	−	−	NA	NA	−	−	+ (had slightly prominent extra-axial spaces on all 3 brain MRIs; last in 2007)	−	−
	Feeding problems	−	+	−	+ in neonatal age	−	+	−	−	+ hx of GER, constipation, eosinophilic esophagitis; now has only intermittent constipation	−	−
	Hearing problems	−	−	−	+	−	+	+	−	−	+	+
	Seizures	−	−	−	−	−	−	−	+	+ infantile spasms resolved (s/p Topomax and ACTH treatment course in the past)	+	−
	Frequent infections	−	−	−	−	−	+	−	−	−	−	−
Cognitive development	ID	+	+ (IQ-Test 2016: 52)	+	NA	+	NA	+	+	+	+	NA
	Behavioral problems	+ unaware of personal boundaries	+ very shy	+ hyperactive and oppositional	−	-	+ hyperactive and oppositional	−	+ sensitive, poor attention seeking skills, poor spacial awareness	+ tantrums, autistic behaviors, hand stereotypies	−	+ high functioning autism
Motor development	Age of sitting independenty	NA	8–9 months	6 months	18 months	9 months	12 months	10 months	15 months	NA	NA	NA
	Age of walking independently	20 months	24 months	30 months	24 months	17 months	24 months	14 months	32 months	24 months	30–36 months	NA
Verbal development	Age of first words	Currently non-verbal	4 years	3 years	16 months	12 months	12 months	30 months	4 years	non-verbal at 15 years	First words unknown, 5 word sentences at 5 years	NA
Other		Epicanthus						Broad uvula, hypoplastic 12th ribs, small bilateral cervical ribs, slight dextroconvex scoliosis, conductive hearing loss	Poor scrotal development, flat feet, congenital vertical talus, chronic constipation	Gastroesophageal reflux (resolved now)	Hypotonia, mildly dilated aortic root, mosaic skin hyperpigmentation	History of headaches and vomiting of unclear etilology, unusual subaortic ventricular septum, bilataral sensorineural hearing loss, kyphosis, gait and balance issues

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, atrial septal defect; CdLS, Comelia de Lange syndrome; ID, intellectual disability; KBGS, KBG syndrome; MRI, magnetic resonance imaging; NA, not assessed; OFC, occipital frontal circumference; SD, standard deviation scores; VSD, ventricular septal defect.

TABLE 2 Comparison of the main clinical features of CdLS and KBG

						Individual 7		Individual 9		Individual 12	
		CdLS	KBG	Individual 3 Single evaluation 7 years 6 months	Individual 4 Single evaluation 9 years	First evaluation 18 months	Last evaluation 6 years 10 months	First evaluation 3 years 3 months	Last evaluation 10 years 4 months	First evaluation 5 years 2 months	Last evaluation 11 years 6 months
Growth	IUGR	+	−	−	NA	−		+		−	
	Postnatal short stature	+	+	−	+	±	−	+	(−)(growth hormone treatment)	−	−
	Microcephaly	+	−	−	−	−	−	+	NA	−	−
Craniofacial features	Brachycephaly	+	+	−	−	+	+	+	−	−	−
	Low anterior hairline	+	±	+	NA	+	+	+	+	+	+
	Frontal bossing	−	+	−	−	−	+	−	−	−	−
	Triangular face	−	+	+	+	−	±	−	+	+	+
	Prominent cheekbones	−	+	+	+	−	+	−	NA	+	+
	Thick eyebrows	+	+	+	+	−	+	−	+	+	+
	Synophrys	+	±	+	+	±	+	+	NA	−	−
	Long eyelashes	+	+	+	−	±	+	+	+	Not long, but prominent	Not long, but prominent
	Depressed nasal bridge	+	−	−	−	±	−	−	−	−	−
	Prominent nasal bridge	−	+	+	−	−	±	−	NA	mild	mild
	Anteverted nostrils	+	+	+	+	+	−	+	+	+	+
	Bulbous nasal tip	−	+	−	−	−	±	−	+	(+)	(+)
	Long smooth philtrum	+	+	+	Long	Long	+	−	+	+	+
	Thin upper lip	+	+	+	−	+	±	−	−	+	+
	Downturned corners of the mouth	+	−	−	−	−	−	−	NA	−	−
	Widely spaced teeth	+	−	+	−	NA	−	−	NA	−	−
	Macrodontia	−	+	−	+	−	−	−	+	+	+
	Micrognathia	+	−	−	−	±	−	−	NA	Mild	Mild

Abbreviations: CdLS, Cornelia de Lange syndrome; NA, not assessed; IUGR, intrauterine growth retardation.

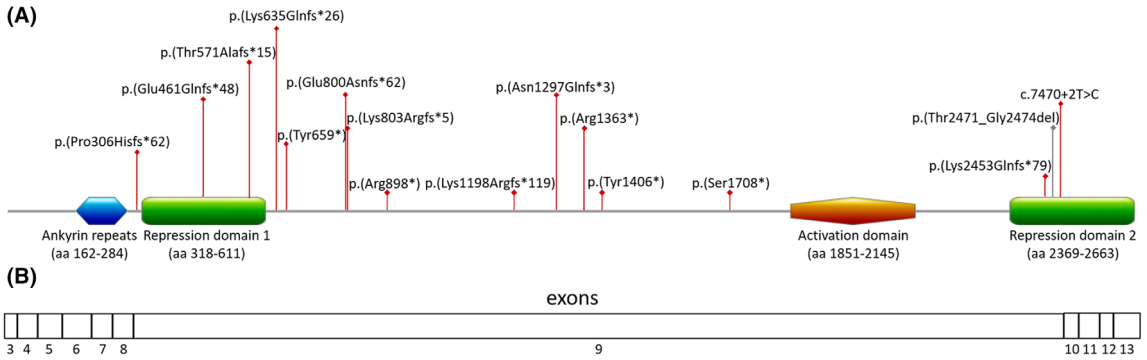

FIGURE 2 Distribution of the ANKRD11 variants at the protein and DNA level. (A) Schematic representation of the ANKRD11 protein and its domains, with relative position of the identified variants (generated with PROSITE MyDomains[37]). The ankyrin repeats are shown in blue, the repression domains in green and the activation domain in orange. Loss-of-function mutations are depicted in red, whereas the in-frame deletion is depicted in gray. (B) Schematic representation of the coding *ANKRD11* exons in scale with the above shown protein. The ankyrin domain is encoded by exons 6–8, the activation and first repression domains by exon 9, and the second repression domain by exons 9–13

overview of the clinical features of these individuals as well as of the differences and commonalities between "classic" manifestations of CdLS and KBGS, with a particular focus on facial dysmorphism.

Individuals 3 and 4 were examined only once by the referring physicians. For both, the facial features at a first evaluation were considered reminiscent of CdLS and, consequently, a clinical diagnosis of CdLS was assigned. After the identification of the *ANKRD11* variants, the clinical features of the individuals were re-evaluated and found compatible with *ANKRD11*-associated KBGS. Individual 12 was examined twice (before and after the molecular diagnosis). No significant phenotypical evolution was observed for this individual but, similarly to Individuals 3 and 4, the re-evaluation of the clinical features appeared consistent with the recently disclosed molecular diagnosis.

Individuals 7 and 9 received a clinical diagnosis of CdLS at an early age. The diagnosis of Individual 7 was mainly driven by the facial features, which included brachycephaly, low anterior hairline, synophrys, long eyelashes, depressed nasal bridge, anteverted nostrils, long philtrum, thin upper lip, and micrognathia. Individual 9 was characterized by intrauterine growth retardation, microcephaly, brachycephaly, low anterior hairline, synophrys, long eyelashes, and anteverted nares. At the latest evaluation, an evolution of the phenotype was observed, primarily related to the shape of the face and the nose. Both subjects developed a triangular shaped face and a bulbous nasal tip. In addition, Individual 7 presented with frontal bossing and prominent cheekbones, while the permanent dentition of Individual 9 featured macrodontia.

3.4 | Molecular findings

Seventeen distinct *ANKRD11* variants were identified in our cohort composed of 23 individuals (Figure 2), including seven out-of-frame deletions (c.915delA, p.(Pro306Hisfs*62); c.1711_1723del, p.(Thr571Alafs*15); c.2398_2401del, p.(Glu800Asnfs*62); c.2408_2412del, p.(Lys803Argfs*5); c.1903_1907del, p.(Lys635Glnfs*26); c.1381_1384del, p.(Glu461Glnfs*48); c.3591_3594del, p.(Lys1198Argfs*119)), two out-of-frame duplications (c.7356dupC, p.(Lys2453Glnfs*79); c.3888dupC, p.(Asn1297Glnfs*3)), five nonsense variants (c.1977C>A, p.(Tyr659*); c.2692C>T, p.(Arg898*); c.4218C>A, p.(Tyr1406*); c.4087C>T, p.(Arg1363*); c.5123C>A, p.(Ser1708*)), one in-frame deletion (c.7411_7422del, p.(Thr2471_Gly2474del)), one splicing variant (c.7470+2T>C) and one deletion encompassing multiple exons (chr16:89,335,426-89,371,803del). All point variants primarily involve exon 9 of *ANKRD11*, hence confirming the role of this exon as mutational hotspot of *ANKRD11*.[17,27] The four amino acids deletion (Individual 8; c.7411_7422del; p.(Thr2471_Gly2474del)) is located in the highly conserved C-terminal repression domain, which is important for proteasome-mediated degradation of ANKRD11.[18] This variant might therefore impair the functional activity of the protein and/or trigger a dominant negative effect upon dimerization with wild type ANKRD11. The remaining loss-of-function variants are instead predicted to activate nonsense-mediated mRNA decay, thereby resulting in *ANKRD11* haploinsufficiency.

Of the mutations herein described, 11 are novel and six have been previously described, namely p.(Glu461Glnfs*48), p.(Lys635Glufs*26), p.(Tyr659*), p.(Glu800Asnfs*62), p.(Lys803Argfs*5), and p.(Arg1363*). The p.(Lys635Glufs*26) variant appears to be particularly frequent in the population of individuals with developmental disorders, as it was already reported in the literature in 10 different families and is also shared by four unrelated individuals of our cohort (Individuals 9, 14, 18, and 19).[19,20,23,24,27,33,38–42]

3.5 | Familial cases

The inheritance of the variants could be verified in 18 individuals. The mutation occurred de novo in 15 individuals and was maternally inherited in Individuals 4, 12, and 13. Detailed clinical data of the mother of Individual 12 are currently not available. The mother of Individual 4 was reported as mildly affected. She presents with short

stature, low weight, deep-set eyes, depressed nasal bridge, large mouth, proximally set thumbs, clinodactyly of the fifth finger and incomplete prono-supination of the elbow. The mother of Individual 13 (Figure 1(I)) received a clinical diagnosis of KBGS and displayed a low anterior hairline, arched and thick eyebrows with synophrys, long eyelashes, myopia, anteverted nostrils, thick alae nasi, a large mouth with thin upper vermilion and thick lower vermilion, macrodontia of central incisors and mild intellectual disability.

4 | DISCUSSION

ANKRD11 plays a pivotal role in the pathogenesis of KBGS and related disorders. Herein we report on 23 individuals carrying 17 distinct variants in *ANKRD11*, thereby expanding the cohort of individuals with mutations in this gene. Reasons for referral of index cases were growth retardation, facial dysmorphism and a variable degree of developmental delay and intellectual disability. All individuals underwent complete physical and dysmorphological evaluation from expert clinical geneticists and all but two (Individuals 13 and 16) received a clinical diagnosis of either CdLS or KBGS. Photographic material was submitted to Face2Gene for an additional clinical evaluation. This database includes an unprecedented amount of phenotypic and genotypic information associated with more than 10 000 diseases and has proven to be a valuable tool for the interpretation of facial features.[36,43,44] Face2Gene assigned a diagnosis of KBGS with high/medium similarity scores to 17 out of 21 individuals for whom photographs were available. The remaining four individuals (Individuals 5, 15, 20, and 23) were not associated to any syndrome with a high probability. Previous studies have proven that the Facial Dysmorphology Novel Analysis technology could match the capabilities of expert clinicians and in some cases also outperform them.[36,43,44] Diagnosis-aiding tools are particularly important for syndromes like KBGS, for which some of the typical and most recognizable clinical features (i.e., macrodontia, delayed bone age, and a bulbous nasal tip/broad nasal bridge) might appear only later in life. Our data confirm the importance of facial analysis technologies as a tool to assist geneticists to assess the most appropriate clinical diagnosis in order to facilitate management and treatment of patients.

The most frequent features reported in our cohort and in the literature comprise intellectual disability, delayed or absent speech, motor delay, behavioral problems and a characteristic facial gestalt. Limb anomalies, delayed bone age, feeding difficulty and visual problems are also frequently observed.[19,20] Number and severity of each of these clinical signs vary within our cohort. The specific combination of features can therefore lead to a broad spectrum of clinical phenotypes, ranging from mild to severe. Previous publications have shown that the severity of the phenotype does not depend on the type or position of the *ANKRD11* variant.[18–20] Comparison of clinical signs of all reported cases of recurrent mutations (in our cohort and in previously reported individuals) as well as of familial cases has confirmed the absence of a linear genotype–phenotype correlation and highlighted the existence of variable penetrance and intra-familial variability.[20] Importantly, the possibility of multiple molecular diagnoses should also be taken into account for an appropriate evaluation of these phenotypes, as they can influence the phenotypical appearance.[45] The majority of our individuals was analyzed by targeted gene panel and we are therefore unable to exclude the presence of additional variants.

Variants in *ANKRD11* have been mainly described in association with KBGS.[5,19,20,27] Accordingly, based on a recent review, 171 out of 228 individuals with *ANKRD11* variants reported in 38 different studies were formally diagnosed as KBGS.[34] However, variants in this gene have also been identified in individuals with neurodevelopmental disorders other than KBGS, namely CdLS and CSS.[23,29–32] These syndromes share some overlapping clinical features that may be difficult to discern from one another.[11,46,47] Eight of the individuals reported here received an initial clinical diagnosis of mild CdLS or atypical CdLS. However, after re-evaluation later in life, a KBGS diagnosis was assigned to five of these individuals. Notably, for two of them, the primary phenotypical differences between the first and the last clinical evaluation were related to the shape of the face and the region of the nose, a finding that is compatible with the physiological progression into adolescence/adulthood. Together with a previously reported CdLS subject with a variant in *ANKRD11*,[30] our cohort points to the existence of an age-dependent phenotypical evolution from CdLS to KBGS from infancy to adolescence, mainly concerning nose and facial contour. Therefore, clinical follow ups are crucial for the assessment of the proper clinical diagnosis.

Importantly, the protein complexes so far associated with CdLS, CSS, and KBGS are all involved in the regulation of transcription and chromatin structure. Epigenetic modifications and transcriptional dysregulation are therefore considered a key molecular feature of these disorders.[6,48] ANKRD11 can control chromatin accessibility and mediate transcriptional regulation upon interaction with histone deacetylases and nuclear receptors.[4,49] The cohesin complex, responsible for the onset of CdLS, is important for gene expression, DNA repair and long-range interactions between distant genomic regions.[50] The CSS-associated SWItch/Sucrose Non-Fermentable (SWI/SNF) complex is well known for chromatin remodeling.[47] Notably, the SWI/SNF complex is known to directly interact with the cohesin loader. In yeast, the SWI/SNF complex recruits the cohesin loader to nucleosome-free regions that the cohesin loader subsequently helps to maintain.[51] A direct interaction between ANKRD11 and these two protein complexes has instead not been reported yet. The substantial functional overlap that characterizes these proteins as well as the direct physical interactions that have been reported for some of them could be found accountable for the shared clinical features observed in individuals with different neurodevelopmental disorders. The increasing accessibility of next generation sequencing technologies will allow the identification of additional variants in *ANKRD11* in individuals with clinical diagnoses different from KBGS. Correspondingly, several variants in *ANKRD11* were identified in individuals with severe undiagnosed developmental disorders and/or intellectual disability.[33,34,52] Also in this cohort, variants in *ANKRD11* have been reported in KBGS subjects but also in individuals with nonspecific

intellectual disability or with CdLS. For this reason, variants in this gene should be ascribed to a more ample group of neurodevelopmental disorders that fall within the category of chromatinopathies rather than to KBGS per se.[53,54] In line with the recently proposed dyadic approach for the description of diagnostic entities,[55] the disease phenotypes observed in association with variants in *ANKRD11* could be defined as "*ANKRD11*-related chromatinopathies" or "*ANKRD11*-related neurodevelopmental disorders."

The growing number of variants in *ANKRD11* and the varying severity of behavioral and developmental phenotypes associated with these variants make the understanding of the causative mechanisms particularly important. Since the levels of *ANKRD11* are tightly regulated during the cell cycle, the most likely pathogenic mechanism is haploinsufficiency.[18] However, analysis of *ANKRD11* transcript levels in cell lines of patients has revealed escape from nonsense-mediated mRNA decay to some extent.[15,18] Furthermore, some variants might also lead to a dominant negative pathogenic mechanism due to a lack of proteasome-mediated degradation of the truncated protein. This proposed mechanism depends on the dimerization between the N-terminal repression domains of the wild type and mutant ANKRD11 when the degradation of the mutant protein is impaired by the disruption of the C-terminal degradation signals.[18] The potential that a greater understanding of the molecular mechanism may lead to eventual therapeutic insights represents an exciting prospect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

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Not applicable.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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