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KBG SYNDROME: AN AUSTRALIAN EXPERIENCE

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Abstract

In 2011, heterozygous mutations in the *ANKRD11* gene were identified in patients with KBG syndrome. Since then, 100 cases have been described with the expansion of the clinical phenotype. Here we present 18 KBG affected individuals from 13 unrelated families, 16 with pathogenic mutations in the *ANKRD11* gene. Consistent features included intellectual disability, macrodontia and the characteristic broad forehead with hypertelorism and a prominent nasal bridge. Common features included hand anomalies, cryptorchidism and a large number of palate abnormalities. Distinctive findings in this series included malrotation of the abdominal viscera, bilateral inguinal herniae in two patients, basal ganglia calcification and the finding of osteopenia in three patients. Nine novel heterozygous variants were found and the genotype-phenotype correlation was explored. This report highlights the need for thorough examination and investigation of the dental and skeletal systems. The results confirm the specificity of *ANKRD11* mutations in KBG and further evidence for this transcription repressor in neural, cardiac and skeletal development. The description of further cases of KBG syndrome is needed to further delineate this condition, in particular the specific neurological and behavioural phenotype.

Key Words

"KBG syndrome", "macrodontia", "intellectual disability", "*ANRKD11"*

INTRODUCTION

Facial dysmorphism, macrodontia, skeletal anomalies and developmental delay have been identified as the major features of KBG syndrome [Herrmann et al., 1975; Brancati et al., 2004]. Clinical diagnostic criteria are based on a review of 50 patients [Skjei et al., 2007]. Bone age examination in childhood was suggested to be removed from diagnostic criteria [Ockeloen et al., 2015].

In 2011 whole exome sequencing in two affected families identified mutations in *ANKRD11* [Sirmaci et al., 2015]. Sanger sequencing found *de novo* truncating *ANKRD11* mutations in three other cases. Four patients had mutations in exon 10 and one family had a mutation at the exon 12 splice site acceptor site according to transcript [NM_001256182.1].

In a recent study 19 reported patients had mutations in exon 10 of *ANRKD11* gene [Ockeloen et al., 2015]. *ANKRD11* was initially hypothesised as a tumour suppressor gene. *ANKRD11* overexpression inhibits transcriptional activation *in vitro* [Zhang et al., 2004]. The transcriptional regulatory domains of the protein include two repressor domains at the N and C termini and an additional activation domain [Zhang et al., 2007]. The ANKRD11 protein is highly expressed in the human brain and localised to nuclei of neurons and glial cells [Gallagher et al., 2015]. ANKRD11 influences the expression of several genes related to neural development, highlighting its association with the neurobehavioral and developmental phenotype in KBG syndrome and providing a mechanism for less common phenotypic associations such as periventricular nodular heterotopia [Oegema et al., 2010]. While

studies on peripheral blood mRNA from a patient with a truncating *ANKRD11* mutation demonstrated incomplete nonsense mediated decay, a dominant negative effect was also hypothesized [Walz et al., 2015]. Similarities between the 16q24.3 microdeletion syndrome and KBG syndrome suggest that these are overlapping entities mediated by *ANKRD11* haploinsufficiency [Willemsen et al., 2010; Goldenberg et al., 2016]. Results from the DDD triome study in the UK found that mutations in *ANKRD11* accounted for around 1% of patients with an undiagnosed developmental delay [Wright et al., 2015]. This finding reflects that KBG syndrome is still an under-recognised clinical condition. Discovery of patients with apparent non-syndromic intellectual disability with *ANKRD11* mutations has led to revised diagnostic criteria for KBG syndrome [Low et al., 2016]. These proposed criteria exclude costovertebral anomalies and delayed bone age, promoting otitis media and hearing anomalies along with seizures, cryptorchidism, feeding problems, palate insufficiency and delayed anterior fontanelle closure.

We report on 18 patients from 13 families with KBG syndrome to further expand the phenotypic and mutational spectrum.

METHODOLOGY

This study includes 18 patients from 13 families referred to genetic services in Australia between 2005 and 2016. Sixteen patients from 11 families had genetic testing. Two other patients who were diagnosed on the basis of clinical criteria did not have genetic testing. Ten patients were clinically diagnosed with KBG syndrome based on the Washington criteria by a clinical geneticist and had Sanger sequencing of *ANKRD11*. Six patients were diagnosed after testing of a customised gene panel for intellectual disability, whole exome sequencing or whole genome sequencing.

Written informed consent was obtained for inclusion in the studies and the use of clinical reports and photographs in accordance with local ethics protocols. All patients underwent standard chromosomal microarray according to local laboratory procedures. Genomic sequences are reported with respect to transcript NM_001256182.1 [GRCh37]. *In silico* prediction of pathogenicity included AlignGVGD, Alamut visual, SIFT and Polyphen. Variants predicted to cause truncation or to affect proper splicing were considered pathogenic.

We reviewed the previously reported individuals with proven mutations or deletions in the *ANKRD11* gene and compared them with our patients.

CLINICAL REPORTS:

The clinical, radiologic and molecular findings are summarised in Tables I-III and Figures 1-7 and in supplementary material.

RESULTS AND DISCUSSION

CLINICAL AND RADIOLOGICAL

Of our 18 patients, all but one met minimum clinical diagnostic criteria albeit with significant inter and intra-familial variability.

Phenotype

Seventeen patients had the facial appearance characteristic of KBG syndrome. This concurred with previous reports [Low et al., 2016, Goldenberg et al., 2015] which showed characteristic facies in 75% of mutation and 89% of deletion cases, including those identified through the DDD study. The commonest dysmorphic features in our patients included ear abnormalities in 16/18 [89%], and the presence of cranial shape anomalies in 15/18 patients [83%]. A prominent nasal bridge was present in 10/18 [56%] patients, while hypoplastic alae nasi were present in 4/18

[22%]. Abnormal philtrum length and morphology was detected in 12/18 patients [66.7%] while 14/18 [77.8%] had hypertelorism.

Ear, Nose and Throat aspects

Hearing loss was reported in 6/18 [33%] compared with 27% in previous cases. Interestingly, a case of severe hearing loss including a sensorineural component was reported in a patient with the recurrent 1903_1907 deletion which was previously associated with a milder hearing loss phenotype [Low et al., 2016]. Patient AU3.2 had unilateral hearing loss since birth, not otherwise specified. The remaining patients had a purely conductive pattern of hearing loss (Table I).

Ten patients of 17 [58.9%] had an abnormality of the palate, which was higher than in previous reports of both mutation and deletion patients [21.1%]. Unrelated patients AU2.1 and AU5.1 had a bifid uvula, and patients 3.1 and 3.3 had an incomplete palate diagnosed endoscopically. The remaining anomaly was presence of a high arched palate.

Dental aspects

Dental findings in this cohort were similar to those previously described and included macrodontia* in 11/12 patients [91.7%], excluding those patients with primary dentition. Other dental abnormalities included premature tooth loss in patients AU1.1, AU3.2 and AU3.3 [16.6%], and crowding in 5/18 [27.8%] patients. Patients AU1.3 and AU8.1 had fusion of the permanent maxillary central incisors.

Hand Anomalies

Hand anomalies were common in 15/18 [83%], higher than in previous reports [70% of mutation and 65.5% of deletion cases]. Brachydactyly and 5th finger clinodactyly [11/18 cases each] were the commonest; while cutaneous syndactyly was identified in 5/18 cases [27.78%].

Neurological and Psychiatric features

Intellectual disability ranging from mild to severe was present in all 18 of our patients, compared with previous reports [97.7%]. Nine patients, including half-brothers AU1.1 and 1.2, had significant language delay out of proportion to performance on standardized cognitive testing. Only one of these patients met DSM-V criteria for autism spectrum disorder. This concurs with the findings of Low et al., which also reported disproportionate language deficits. Only 3 of our cohort had seizures diagnosed at the time of writing (16.7%), compared with 28.3% of patients with *ANRKD11* mutations reported previously and 30.3% of patients with a contiguous gene deletion at the 16q23 region. The previously identified pattern of seizures improving with age [Ockeloen et al., 2015] was observed patient AU5.1, who was seizure free by the age of 6. Six patients in this series underwent MRI brain scanning. Patient AU5.1 had an MRI at the age of 4 showing small arachnoid and pineal cysts, while patient 4.1 had a low lying conus medullaris, partial agenesis of the corpus callosum, and choroid plexus cysts. Patient AU1.2 had small optic nerves and volume loss with a developmental venous anomaly in the frontal lobe. Patient AU11.1 had an MRI showing a dilated left sided endolymphatic sac and arachnoid cysts overlying the cerebellar hemispheres. MRI for patient AU13.1 was markedly abnormal and showed bilateral T2 hyperintensities, caudate swelling and high signal and calcific foci in the periventricular white matter and right thalamus. Arachnoid cysts were also present. No environmental cause was found to explain these findings and whole exome sequencing did not reveal an alternative genetic explanation. Results from the MRI brain of patient 12.1 were unremarkable.

Two patients in this series had the newly recognised feature of a large anterior fontanelle and delayed closure.

Significant behavioural and psychiatric abnormalities are increasingly recognised in this syndrome [Ockeloen et al., 2015] and were evident in 50% of our patients. Physical aggression was reported in 3 of our patients [16.7%] along with reports of temper tantrums and inflexible personality traits in patients 3.1 and 8.1 and a clinical diagnosis of Bipolar Affective Disorder in patient 1.3. There did not appear to be a correlation between behaviour with the degree of intellectual disability, and further research is needed to delineate the behavioural profiles manifest in KBG syndrome. Previous reports suggested major behavioural problems in 70% of those with a mutation and 53% with a deletion of *ANKRD11*. Visual anomalies were present in 10/18 patients [56%], with refractive errors or astigmatism, 2 of whom had concurrent strabismus. Lowe et al reported a similar frequency of refractive errors in their study [Low et al., 2016].

Cardiac features

Congenital heart disease is a feature of mutation positive KBG syndrome, present in 4/18 [22%] unrelated patients as reported previously. Patient AU3.3 had an atrial septal defect and ventriculoseptal defect [VSD] repairs in childhood while Patient 1.2 had a VSD and patent ductus arteriosus on an echocardiogram in infancy. Patient 6.1 had a primum atrial septal defect and a cleft mitral valve while patient 12.1 had a persistent patent ductus arteriosus.

Genitourinary features

Cryptorchidism was identified previously in 16/17 [97%] of patients s with a mutation and in 2/10 with a deletion of *ANKRD11*. 3/7 of our male patients required operative management to correct cryptorchidism in childhood, reiterating that this is a common phenotypic feature. One patient had precocious puberty, a finding reported in 5/39 patients in a recent review [Goldenberg et al., 2016].

Skeletal aspects

The presence of skeletal features including stature <3rd centile, delayed bone maturation and costovertebral anomalies form 3 of the major diagnostic criteria for KBG syndrome [Skjei et al., 2007]. Only 8/18 of our patients [44%] had postnatal short stature <3rd centile, with an additional patient on the tenth centile, and two patients had intrauterine growth restriction [IUGR]. The remaining patients ranged between the 50th and 75th centile, indicating that postnatal short stature may not be as common in Australian patients with this syndrome.

The costovertebral anomalies we observed were variable and generally mild. Four of 16 patients had a normal survey other than the presence of short metacarpals and metatarsals, while an additional three patients had only mild scoliosis or lordosis. Patient AU4.1 had bilateral coxa valga, while patient AU7.1 had narrow spaces in the thoracic spine and symphalangism in toes 3-5 along with congenital bilateral hip dislocation. Patient AU6.1 had marked pectus excavatum and an exaggerated lumbar lordosis. The rates of vertebral abnormalities were higher in the patients published by Ockeloen and Sirmaci [14/26, 53.85%], and hip abnormalities were more common [16%]. Delayed bone maturation was evident in 5/10 paediatric patients and osteopenia was also identified in 3 children. Our findings suggest wide variability in the skeletal manifestations of this condition, and that costovertebral abnormalities may be less prevalent than previously thought.

New features identified

Additional findings in our patients included aberrant abdominal vessels and malrotation of the abdominal viscera in patient AU4.1, who required a PEG tube for feeding. She also had ectopic kidneys identified on ultrasonography. A malrotated bowel requiring corrective surgery at 10 weeks was also identified in patient AU12.1.

Novel skeletal findings included the presence of clavicular pseudoarthrosis in a patient and the presence of bilateral cervical ribs. Osteopenia was also identified in 3 paediatric patients, including a male patient 1.2 at the age of 5 years, and two female patients 2.1 and 18.1 at the ages of 13 and 6 years respectively. The unusual MRI appearance in patient AU13.1 of widespread calcific changes and basal ganglia abnormalities did not have an identified secondary cause. Another unique finding was of microphthalmia in the same patient and corneal clouding described in an infant. Two patients were identified as having growth restriction *in utero*.

To our knowledge, none of these findings have been reported previously in patients with an *ANKRD11* mutation or deletion.

There was no consistent phenotype-genotype correlation.

Intrafamilial observation

Family 1 included two half-brothers who had similar facial features brachydactyly and abnormalities of the palate. Both had significant learning difficulty, with expressive and receptive language impairment and behavioural problems. The older boy, assessed at age 7, had a history of cryptorchidism and bilateral hearing loss as well as macrodontia. The younger half-brother was a dichorionic diamniotic twin born prematurely at 27 weeks, At the age of four years he had growth delay, osteopenia, and his echocardiogram showed a patent ductus arteriosus and ventricular septal defect. His clinical picture was confounded by complications of prematurity including failure to thrive and grade IV retinopathy. Their mother had a similar facial appearance. She was shorter than her sisters. She had a mild scoliosis and fusion of the central incisors with macrodontia. She had a history of learning difficulty and bipolar affective disorder. The recurrent point mutation c.1903_1907del

[g.89351043_89351047del pLys635GInfs*26] segregated in these three family members.

Family 2 had three generations of affected individuals sharing similar dysmorphic features, nasal speech with palate incompetence, brachydactyly and intellectual disability. Individual 3.1, examined at age 13, had macrodontia, a unilateral strabismus along with behavioural issues including aggression and inflexibility. Her height was on the 75th centile, while her mother [3.2] and grandmother [3.3] had heights on the 3rd centile. 3.2 had the additional features of congenital right sided hearing loss and a hypoplastic 12th rib, while individual 3.3 had a childhood history of repaired atrial and ventricular septal defects. 3.2 and 3.3 had premature decay of permanent dentition. A novel nonsense mutation [g.89348544 C>T p.Try1469*] segregated with KBG syndrome in this family.

Family 12 came to attention with the clinical diagnosis of KBG syndrome in individual 12.1, who had conductive hearing loss, strabismus, intellectual disability with language delay, and dysmorphism including brachydactyly, syndactyly, a webbed neck, and a broad forehead. Other features included a malrotated bowel at 10 weeks of age, along with delayed bone age and a patent ductus arteriosus. A novel missense mutation was detected in exon 10 of ANKRD11 [g.89349508C>T; p.Gly1148Ser], and identified in her father [12.2] on segregation studies. 12.2 did not meet diagnostic criteria although he had some consistent facial features and a history of learning difficulty. He had no apparent skeletal and cardiac anomalies and his height was at 90th centile.

Revised Diagnostic Criteria

Recently proposed revisions to the Skjei criteria are published previously [Skjei et al., 2007; Low et al., 2016].

We agree with the authors that stature below the 10th centile is more appropriate than stature below the 3rd centile as a diagnostic criterion. However, 10/18 of our patients [66%] had a stature on or above the tenth centile, with two patients on the 75-90th centile. This suggests that short stature is not as prevalent in Australian patients with KBG syndrome. We also agree with the removal of costovertebral anomalies from the diagnostic criteria as we found these to be variable and mild, as reported by others [Low et al., 2016; Goldenberg et al., 2016]. We agree with the inclusion of palate abnormalities as a diagnostic aid, as these were found in 58% of our patients and 26% overall. Most of our patients had typical hand anomalies [83.3%] and neurological features including learning disability [100%] and we recommend that these remain as principle features in standard diagnostic criteria.

Our patients deviated from reported data in the low incidence of seizures [16.7%], although EEGs were not performed in most. The low frequency of autism spectrum disorder reflects the findings in other report [Goldenberg et al., 2016] but it represents a departure from the previous estimates of 25% [Low et al., 2016] and 47% [Ockeloen et al., 2015].

MOLECULAR RESULTS (Table III)

Mutations in the *ANKRD11* gene were reported with respect to genome assembly GRCh37/Hg19 and transcript NM001256182.1. The UCSC browser was used in conjunction with the Alamut database. SIFT, Polyphen and AlignGVGD were used for in silico prediction of pathogenicity for the novel missense mutation reported in patients AU12.1 and 12.2. Five nonsense, four frameshift mutations, one splice site mutation and one missense mutation were identified. Nine novel mutations were identified. The p.Glu169* variant in AU2.1 truncates the protein from the first (amino acids 167-196) of the four ANK domains of ANKRD11. It is the closest variant to the

N-terminal of the protein reported to date. It has been shown that ANKRD11 protein homodimerizes through the ANK domains located at the N-terminal. Since all other mutations reported to date left the N-terminal of the protein intact, it has been hypothesized that the truncated ANKRD11 may bind the normal ANKRD11 via its N-terminal, potentially impairing the function of the normal copy. The presence of typical manifestations of KBG syndrome in our patient 2.1 in whom complete nonsense mediated mRNA decay was expected supported *ANKRD11* haploinsufficiency in causation of KBG syndrome. This is an interesting finding that is against the hypothesis of dominant negative mechanism acting in the pathogenesis of KBG syndrome.

There was no observed correlation between phenotype and the site or domain at which protein truncation was predicted. (Figure 6) Features and severity did not appear to correlate with mutations in the *de novo* form although the father AU12.2 did not meet clinical criteria and had a pathogenic mutation detected on segregation studies, suggesting a milder effect conferred by this missense mutation. The findings confirm specificity of *ANKRD11* mutations in KBG syndrome as well as allelic heterogeneity.

CONCLUSION

Our 18 Australian patients with KBG syndrome including 16 with a mutation in *ANKRD11* highlights several key aspects of KBG syndrome, including the ongoing clinical utility of the diagnostic criteria with potential revisions accounting for normal stature and infrequency of costovertebral anomalies. Diagnosis may be delayed in the proband and examination of parental dentition may provide an essential clue.

Language delay is severe and out of proportion to deficits in other domains and the behavioural features do not appear to correlate with the degree of intellectual

disability. We recommend echocardiogram, renal ultrasonography, ophthalmic and hearing assessments and good dental care along with formal developmental assessments and appropriate early intervention. We also recommend bone density measurement because of increased frequency of osteopenia (16%). There is significant allelic heterogeneity in this condition. The results confirm the specificity of *ANKRD11* mutations in KBG and provide further evidence for this transcription repressor in neural, cardiac and skeletal development. Further mutation testing of clinically affected individuals will assist in broadening our understanding of the phenotype and genotype-phenotype correlations, in particular the skeletal and dental manifestations of this syndrome as well as the neurobehavioral phenotype.

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Figures and Legends.

1. Family 1 Pedigree
2. Family 3 Pedigree
3. Facial photographs.

   Row 1: Family 1. Image 1-2: Patient 1.1 demonstrating broad forehead, triangular facies and thick upper vermilion. Image 3: Patient 1.2 [right] pictured with unaffected dizygotic twin brother. Image 4-5: Patient 1.3 demonstrating downslanting palpebral fissures, fused central incisor and short, webbed neck.

   Row 2: Image 1-2: Patient 2.1 demonstrating hypertelorism, macrodontia, bulbous nasal tip and dysplastic left ear. Image 3: Patient 9.1 showing hypertelorism, long philtrum and broad forehead. Image 4-5: Patients 3.1-3.3 taken in 2002. Note the photograph of infant 3.1 which demonstrates brachyturricephaly.

   Row 3: Image 1-2: Patients 3.1 and 3.2 in 2014 demonstrating characteristic craniofacial shape. Image 3: Patient 3.1 demonstrating macrodontia. Image 4-5: Patient 4.1 in infancy showing dysmorphic features including long philtrum, upslanting palpebral fissures and broad forehead. Image 6-7: Patient 5.1at age 15 demonstrating broad brows, retrognathia and broad nasal root. Image 8: Patient 7.1demonstrating a fused central incisor.

   Row 4: Patient 10.1 photographed at 3 months, 41/2 years, 9, 11 and 15 years.
4. Figure, ANKRD11 protein domains demonstrating sites of protein truncation and alteration
5.

Table I Clinical features in 18 patients

6. Table II Summary of clinical features in KBG syndrome.
7. Table III Mutations in KBG syndrome

CASE	1	2	3	4	5	6	7	8	9
FAMILY	1	1	1	2	3	3	3	4	5
	AU1.1	AU1.2	AU1.3	AU2.1	AU3.1	AU3.2	AU3.3	AU4.1	AU5.1
Gender	M	M	F	F	F	F	F	F	M
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Thai	Caucasian
Birth measurements, weight, length and OFC (in centiles)	[10-50th], [50th], [50th]	[50-90th], NK, [10 - 50th]	NK	[50th], [50-90th], [50 - 90th]	[<10th], [<10th], [<10th]	NK	NK	[10-50th], NK, [50th]	[10-50th] [50-90th], [<10th]
Current Height (in centiles)	25th	3rd	50th	25-50th	75th	3rd	3rd	1st	25th
Current OFC	50th	2-50th	50-98th	50th	50th	50th	5-98th	NK	50th
Perinatal Issues	pre-eclampsia	DCDA twin NICU - FTT	-	Antepartum haemorrhage	IUGR, feeding difficulty	-	NK	IUGR, conjugated hyperbilirubinemia	-
Craniofacial	High forehead Triangular face Short neck	Brachyturricephaly High forehead	Triangular face Short, webbed neck	Broad forehead Triangular face	Broad forehead	Brachycephaly Broad forehead Midface hypoplasia	Broad forehead	Triangular face	Triangular face Small, retrognathic chin

Dysmorphic Features	Ptosis Downslanting palpebral fissures Overfolded helix Low anterior hairline Thick vermillion Long flat philtrum	Downslanting palpebral fissures Anteverted nares High nasal bridge Externally rotated ears Patchy hair loss Thick vermillion Long philtrum	Downslanting palpebral fissures Hypertelorism High nasal bridge Anteverted, low set ears Low anterior hairline Synophrys Thick vermillion	High nasal bridge Bulbous nasal tip Flat left pinna Frontal upsweep Sparse hair Everted upper, thick vermillion Flat philtrum	Hypertelorism Left sided strabismus Anteverted nares High nasal bridge Thin nasal tip Dysplastic left ear Sparse hair Synophrys Thick vermillion Short philtrum	Hypertelorism High nasal bridge Thin nasal tip Simple ears Synophrys Thin vermillion Short philtrum	Ptosis Hypertelorism Thin vermillion Short philtrum	Upslanting palpebral fissures Hypertelorism Anteverted nares Bulbous nasal tip Low set ears, Pits on lobes Bushy brows Long philtrum	Hypertelorism Short upturned nose Anteverted nares High, broad, prominent nasal bridge Prominent ears Low anterior hairline Broad brows flared laterally Synophrys Long philtrum
Development	Severe language delay	Global DD Severe language delay	Learning difficulty	Global DD Severe language delay	Global DD language delay	In lower class at school	learning difficulties	Global DD	Global DD moderate to severe language delay
Neurological features	ADHD	Hypotonia Poor sleep MRI brain: small optic nerves Developmental venous anomaly left frontal lobe	Hyperreflexia Bipolar affective disorder	ADHD Anxiety	ADHD aggressive, inflexible, physical outbursts	-	-	MRI brain: low lying conus medullaris partial agenesis corpus callosum choroid plexus cyst	Seizure onset age 3 Seizures ceased age 6 Autism Spectrum Disorder ADHD Significant behavioural MRI brain - small arachnoid and pineal cysts
Vision	Normal	Retrolental fibrosis Grade IVb ROP [blind]	Myopia Astigmatism, Night blindness	Astigmatism.	Strabismus Refractive error	Normal	Normal	Hypermetropia, Glasses Corneal lesions	Refractive error
Hearing	Bilateral hearing loss NOS	-	-	-	-	Right sided hearing loss since birth	-	Severe mixed hearing loss and enlarged vestibular aqueducts	-
Palatal irregularity	high arched and narrow	bifid uvula	high arched	-	nasal speech, palate incompetent	nasal speech, palate incompetent	nasal speech, palate	-	Bifid uvula, slightly high palate

							incompetent		
Dental Features	Macrodontia crowding premature loss of permanent teeth	Primary dentition	Macrodontia fused central incisors	Macrodontia	Macrodontia	Macrodontia premature loss of permanent teeth	Macrodontia premature loss of permanent teeth	primary dentition	Macrodontia malposition crowding
Other systemic features	Bilateral cryptorchidism	Ventricular septal defect and patent ductus arteriosus on echocardiogram	-	Recurrent intussusception Psoriasis	-	Hypothyroidism	Repaired ventricular septal defect and atrial septal defect	Ectopic kidneys Abdominal viscera malrotation and abnormal vessels, PEG fed	Normal echocardiogram Bilateral inguinal hernia repair Bilateral orchidopexy for undescended testes FTT, GORD from early infancy, settled by 6 months
Skeletal Features	Delayed bone maturation Normal survey	Delayed bone maturation Mild osteopenia Otherwise normal survey	Minor thoracolumbar scoliosis	Mild thoracic scoliosis Mild osteopenia	Normal skeletal Survey	Hypoplastic 12th rib	Degenerative lumbar spine, convex right scoliosis, hyperostosis frontalis interna	Bilateral coxa valga	Mild thoracic scoliosis convex right
Hand and foot anomalies	Bilateral cutaneous 2-3 finger and toe syndactyly	5th finger brachydactyly	5th finger contracture	Bilateral short 4th, 5th metacarpals	5th finger clinodactyly	Brachydactyly 5th finger clinodactyly	Brachydactyly	fixed flexion 5th fingers, bilateral clinodactyly	Bilateral 2-3 and 3-4 cutaneous toe syndactyly
Meets clinical criteria	YES	YES	YES	YES	YES	YES	YES	YES	YES

CASE	10	11	12	13	14	15	16	17	18
FAMILY	6	7	8	9	10	11	12	12	13
	AU6.1	AU7.1	AU8.1	AU9.1	AU10.1	AU11.1	AU12.1	AU12.2	AU13.1
Gender	M	F	F	F	M	M	F	M	F
Age at Assessment	15	28	34	4	15	12 months	6	33	4 and 6
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Pacific Islander
Delivery	SVD, term, suctioning and oxygen	38 weeks, SVD	NK	40 weeks, SVD	SVD at 40 weeks, fetal distress and meconium liquor	29 weeks, emergency LSCS	SVD at 40 weeks	NK	32 weeks LSCS for failure to progress
Birth measurements, weight, length and OFC (in centiles)	[50th] [>50th] [>90th]	[10th], NK, NK	NK	[10th], [10th], [3rd]	[<10th], [50th] [50th]	[25th], NK, [25th]	[<10th], NK, NK	NK	[90th],NK, [50th]
Current Height	1st	10th - 25th	<3rd	10th	Height <3rd and improved to 25th with growth hormone	Corrected for prematurity, 10th	10-25th	75th – 90th	Height <3rd, no response to growth hormone
Current Head Circumference (OFC, in centiles)	2-50th	2nd	2nd	10th	50th	25th	50th	50th	2-50th
Perinatal Issues	Difficulty feeding, reflux, hypotonia	Maternal smoking exposure Congenital bilateral hip dislocation	NK	-	Advanced maternal age Respiratory distress	IUGR, pre-eclampsia Jaundice patent ductus arteriosus TPN required	-	NK	Seizures
Dysmorphic Features									
Craniofacial	Round face	Brachycephaly	Pointed chin Short neck	Large anterior fontanelle with delayed closure Triangular face	Brachycephaly Triangular face, micrognathia Short neck	Large anterior fontanelle with delayed closure Turricephaly	Webbed neck	Broad forehead	Short neck
Dysmorphic Features	Upslanting palpebral fissures Hypertelorism Strabismus Prominent nasal bridge Hypoplastic alae nasi Low set, protruding ears Low hairline anteriorly Bushy eyebrows Micrognathia Thin upper vermillion	Horizontal Palpebral fissures Relative hypertelorism High nasal bridge Bulbous nasal tip Hypoplastic alae nasi Simple helices Wild hair Low hairline Broad eyebrows Synophrys	Upslanting palpebral fissures Hypertelorism Asymmetric nares High nasal bridge Bulbous nasal tip Hypoplastic alae nasi Anteverted dysplastic ears Bushy eyebrows Thick vermillion	Hypertelorism High nasal bridge Bulbous tip Dysplastic ears Pre-auricular pits Synophrys Thin vermillion Long philtrum	Epicanthic folds Hypertelorism Bulbous nasal tip Low set, posteriorly rotated ears, Left overfolded helix; Right anteverted Thick hair Low posterior hairline Broad/bushy eyebrows Thin vermillion Long, flat philtrum	Hypertelorism Anteverted nare Thin vermillion Long, flat philtrum	Long Palpebral fissures Ptosis Bilateral strabismus repair Anteverted nares Bulbous tip Low set , prominent ears Low hairline Broad/bushy eyebrows Thin vermillion	Low columella Simple helices	Long Palpebral fissures Microphthalmia Hypertelorism Prominent columella Bulbous tip Hypoplastic alae nasi Prominent ears Low hairline Broad/bushy eyebrows Synophrys Thin vermillion Flat philtrum
Developmental Hx	Gross motor delay Stutter Nocturnal enuresis mild-moderate intellectual disability	Mild intellectual disability	Mild global developmental delay	Mild global developmental delay IQ 70	Mild DD Language delay	Borderline at 12 months corrected	Fine motor difficulty Language delay	No delay Learning difficulty at school	Expressive and receptive language delay Mostly non-verbal at 6, and signing
Neurological features	nil	moody, aggressive, difficult behaviour	nil	ADHD	ADHD	Hypotonia ADHD MRI brain and inner ear: dilated left endolymphatic sac; arachnoid cysts	Hypotonia	-	Neonatal seizures MRI brain: Bilateral T2 hyperintensities, Multiple calcific foci in brain, arachnoid cyst, germinolytic cysts

Hand and foot anomalies	Brachydactyly	Short tubular bones in fingers	Hand and foot anomalies	Brachydactyly		Brachydactyly
Meets clinical criteria	YES	YES	YES	YES	YES	YES

NA – not available or applicable

NK – not known

NAD – nil abnormalities detected

ND – not done

g– grams

cm – centimetres

SVD – spontaneous vaginal delivery

FTT – failure to thrive

GORD – gastro-oesophageal reflux disease

FRAX – Fragile X

DMacrodontia – central incisor width >10mm in males and >9.7mm in females

DROP – Retinopathy of Prematurity

– developmental delay

	CASES	%	ANKRD11 mutations - Previously Reported	%	Deletion	%	Overall	%
GENDER								
Male	7/18	38.89%	39/78	50.00%	19/33	57.58%	65/129	50.39%
Female	11/18	61.11%	39/78	50.00%	14/33	42.42%	64/129	49.61%
FACIAL APPEARANCE								
Gestalt	17/18	94.44%	59/78	75.64%	31/33	93.94%	105/129	81.40%
Cranial size/ shape anomaly	**15/18**	**83.33%**						
Brachy/turricephaly	5/18	27.78%						
Broad/round/triangular	13/18	72.22%						
Hypertelorism/telecanthus	14/18	77.78%						
Low hairline	8/18	44.44%						
Broad/bushy eyebrows	8/18	44.44%						
Synophrys	7/18	38.89%						
Strabismus	3/18	16.67%						
Ptosis	3/18	16.67%						
Any Ear Anomaly	16/18	88.89%						
Upturned/anteverted nares	8/18	44.44%						
High/prominent bridge	10/18	55.56%						
Bulbous tip	8/18	44.44%						
Mouth and Palate								
Thick vermillion	6/18	33.33%						
Thin vermillion	8/18	44.44%						
Long/flat/hypoplastic philtrum	12/18	66.67%						
Palatal irregularity	**10/17**	**58.82%**	15/71	21.13%	7/33	21.21%	32/121	26.45%
DENTAL								
Macrodontia	**11/12**	**91.67%**	67/81	82.72%	17/26	65.38%	95/119	79.83%

Fused incisors	2/18	11.11%						
Oligodontia	1/18	5.56%						
Crowding	5/18	27.78%						
Premature Decay	3/18	16.6%						
Cleft teeth	0/12	0.00%						
Other dental, total	5/18	27.78%						
HAND ANOMALIES - ALL	**15/18**	**83.33%**	50/71	70.42%	19/29	65.52%	84/118	71.19%
Brachydactyly	11/18	61.11%						
5th finger clinodactyly	11/18	61.11%						
Syndactyly	5/18	27.78%						
NEUROLOGICAL								
Delayed fontanelle closure	2/18	11.11%	7/32	21.88%			9/50	18.00%
Hypotonia	4/18	22.22%						
Intellectual disability	**18/18**	**100.00%**	80/82	97.56%	29/30	96.67%	127/130	97.69%
Seizures	3/18	16.67%	17/60	28.33%	10/33	30.30%	30/111	27.03%
Autism Spectrum Disorder [ASD]	1/18	5.56%	21/97	21.65%			22`/115	19.13%
ADHD/significant behavioural issues	10/18	50.00%	50/71	70.42%	17/32	53.13%	77/121	63.64%
Abnormal MRI/CT brain findings	**5/6**	**83.33%**						
Any visual abnormality	10/18	55.56%						
Hearing loss	6/18	33.33%	19/71	26.76%	8/32	25.00%	33/121	27.27%
Congenital heart disease	4/18	22.22%	14/71	19.72%	8/33	24.24%	26/122	21.31%
Cryptorchidism	3/7	42.86%	11/26	42.31%	2/10	20.00%	16/43	37.21%
Inguinal hernia	3/18	16.67%	2/51	3.92%			5/69	7.25%
SKELETAL ANOMALY, any	**14/18**	**77.78%**						
Stature <3rd percentile	8/18	44.44%	39/82	47.56%	17/32	53.13%	64/132	48.48%
Delayed bone maturation	5/10	50.00%	15/36	41.67%	3/16	18.75%	23/62	37.10%
Accessory ribs	1/16	6.25%						
Abnormal vertebra shape	2/16	12.50%	14/26 [Ockeloen]	53.85%	1/14	7.14%	17/56	30.36%
Osteopenia	3/16	18.75%						
Hip anomaly	2/16	12.50%						
Kyphosis/scoliosis	5/18	27.78%						
Clavicular pseudoarthrosis	1/16	6.25%						
Hypoplastic ribs	1/16	6.25%						

Table 3: Molecular Findings

Patient	1.1	1.2	1.3	2.1	3.1	3.2	3.3
Codon	c.1903_1907 del	c.1903_1907 del	c.1903_1907 del	c.505G>T	c.4406G>A	c.4406G>A	c.4406G>A
Genomic coordinates	g.89351043_89351047del	g.89351043_89351047del	g.89351043_89351047del	g.89357129C>A	g.89348544 C>T	g.89348544 C>T	g.89348544 C>T
Protein	pLys635GInfs*26	pLys635GInfs*26	pLys635GInfs*26	p.Glu169*	p.Trp1469*	p.Trp1469*	p.Trp1469*
Exon	10	10	10	7	10	10	10
Effect	Frameshift	Frameshift	Frameshift	nonsense	nonsense	nonsense	nonsense
Novel/known	known	known	known	Novel	Novel, Inherited	Novel, Inherited	Novel
Sequencing test	Single gene	Single gene	Single gene	Single gene	Single gene	Single gene	Single gene

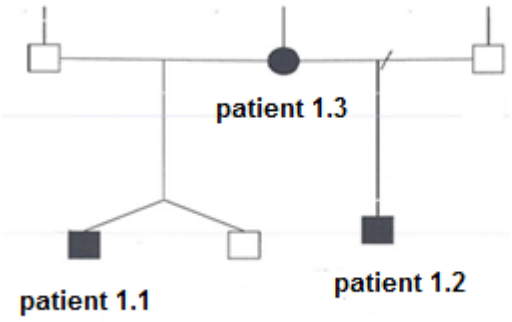
Patient	4.1	5.1	7.1	8.1	9.1	11.1	12.1	12.2	13.1
Codon	c.1903_1907del	c.1173C>G	c.7471A>C	c.6409_6410del	c.3224_3227delCTTT	c.7216C>T	c.3442G>A	c.3442G>A	c.6472G>T
Genomic Coordinates	g.89351043_89351047del	g.89351777G>A	g.89341601T>G	g.89346540_89346541del	g.89349723_89349726delAAAG	g.89346734G>A	g.89349508C>T	g.89349508C>T	g.8934647
Protein	p.lys635GLnfs*26	p.Tyr391*	?	p.Ser2137Profs*9	p.Glu1075Glyfs*242	p.Gln2406*	p.Gly1148Ser	p.Gly1148Ser	p.Glu2158*
Exon	10	10	Splice site acceptor exon 11	10	10	10	10	10	10
Effect	Frameshift	nonsense	Splice site	Frameshift	Frameshift	Nonsense	Missense	Missense	Nonsense
	known	Novel, de novo	Novel, de novo	Novel, de novo	Novel, de novo	Novel, de novo	Novel, inherited	Novel	Novel, de n
Sequencing test	Intellectual disability panel	Single gene	Single gene	Single gene	WES	WGS	Intellectual Disability panel	Segregation	WES

Reported with respect to transcript NM001256182.1 and genome assembly GRCh37/HG19

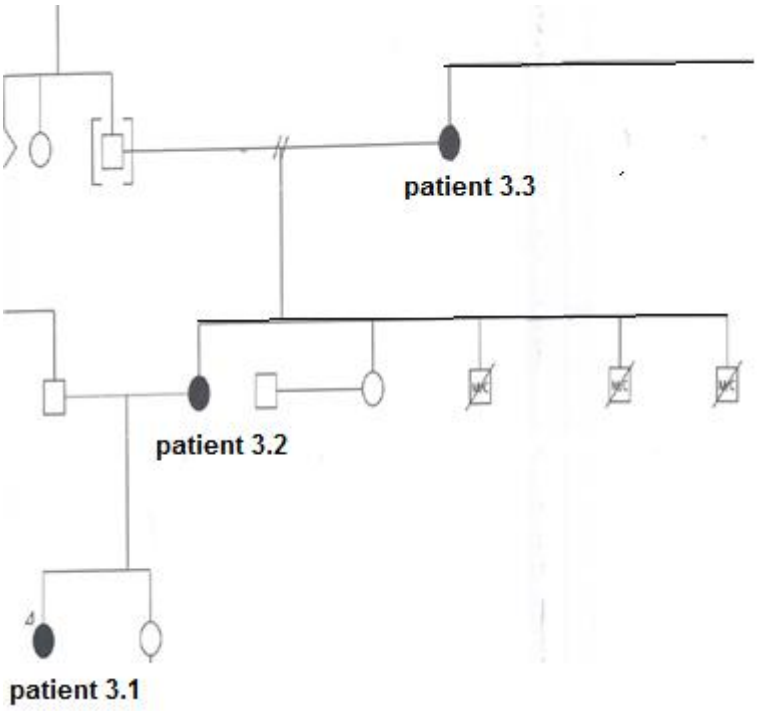
WES = whole exome sequencing

WGS = whole genome sequencing

Patients 6.1 and 10.1 have not yet undergone genetic testing



1Pedigree F1 .



2PedigreeF2 .

3KBG facial photographs .

LIVERPOOL HOSPITAL

NSW GOVERNMENT Health
South Western Sydney Local Health District

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LG-01-0794

18th October 2016

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Son
Daughter

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I understand that NO identifying information, such as name and address, will be published

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Witness J.Webb JENNIFER WEBB (MOTHER)

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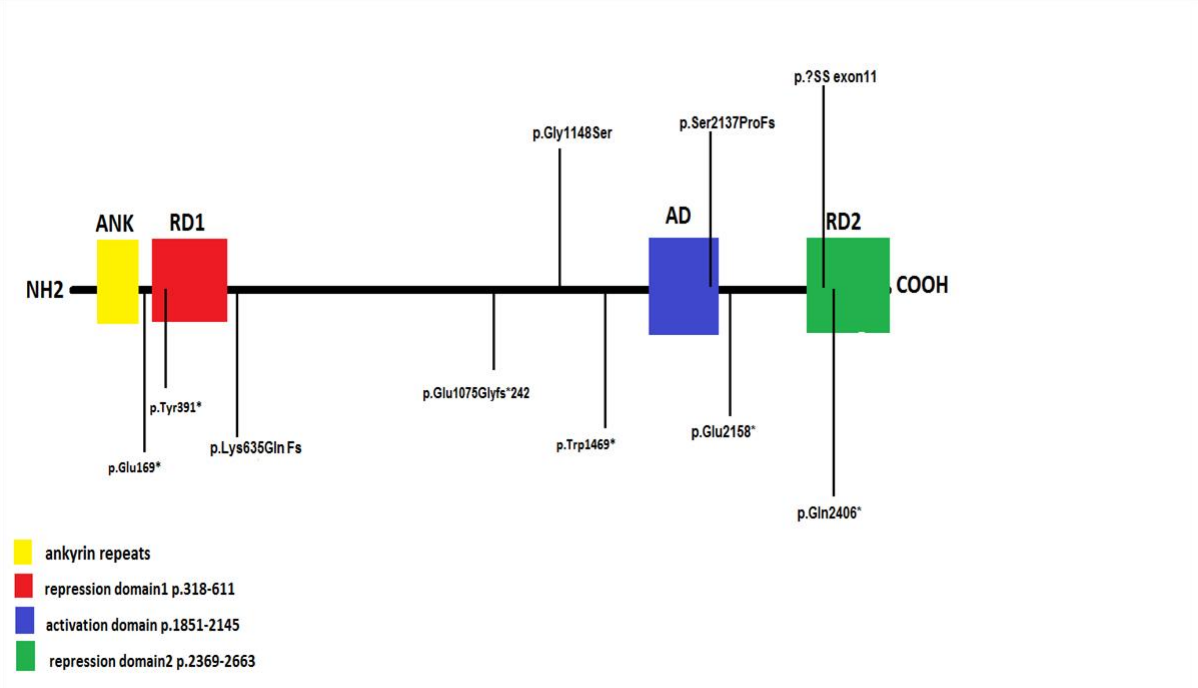

7ANKRD_protein_domains .