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Supplemental Data

Mutations in *ANKRD11* Cause KBG Syndrome, Characterized by Intellectual Disability, Skeletal Malformations, and Macrodontia

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Table S1. Phenotypic Features of All Study Subjects with KBG Syndrome

KBG syndrome[a]	Family 1			2-1	3-1	4-1	5-1	6-1	7-1	8-1	9-1	10-1	Literature total 59 pts[B] (%)[1–18]
	II-1	II-2	I-1										
Country	Turkey	Turkey	Turkey	Turkey	Turkey	Italy	Italy	Italy	Italy	Italy	Italy	**Italy**	
Familial[e] or Simplex (F/S)	F	F	F	S	S	S	S	S	S	F	F	**S**	
Sex (M/F)	M	M	M	M	M	M	M	M	M	F	M	M	38/21
Age at diagnosis/current age	16y/25y	13y/22y	41y/46y	9y/16y	6y/11y	14y/21y	9y/17y	14y/17y	11y/31y	42y/49y	9y/16y	9y/16y	
Postnatal short stature[e]	+	+	+	+	-	+	+	-	-	+	-	-	36/47 (77)
Developmental delay/ mental retardation[d,e]	+	+	+	+	+	+	+	+	+	mild	mild	Mild	55/59 (93)
Craniofacial findings[e]													
Brachycephaly	-	-	+	+	+	+	+	-	+	-	+	+	29/40 (73)
Distinct shape of face (round or triangular)	+	+	+	+	+	+	+	+	+	-	-	+	39/50 (78)
Wide eyebrows/synophrys	+	+	+	+	+	-	+	+	+	-	+	+	30/50 (60)
Hypertelorism	-	+	+	-	+	+	+	+	-	-	-	+	22/40 (55)
Ptosis	+	+	+	+	-	+	+	+	+	+	-	-	10/31 (32)
Anteverted nostrils	+	+	+	+	+	+	+	+	-	-	+	+	46/53 (87)
Prominent nasal bridge	+	+	+	-	-	+	+	+	+	+	+	+	51/58 (88)
Dental findings													
Macrodontia of upper central permanent incisors*	+	+	+	+	+	+	+	+	+	c	+	+	54/55 (98)
Oligodontia	-	+	-	+	+	+	-	-	-	+	-	-	22/38 (58)
Skeletal abnormalities[e]													
Abnormal vertebrae	-	+	+	NA	-	+	-	+	+	+	-	-	37/52 (71)
Cervical ribs	+	-	-	+	-	+	-	+	-	-	-	+	23/48 (48)
Short tubular bones of hands	+	+	+	+	NA	+	+	+	NA	-	+	+	34/51 (67)
Delayed bone age[e]	+	+	NA	+	NA	NA	+	-	+	NA	+	+	20/38 (53)
Other Findings													
Abnormal EEG/seizures*[d]	+	+	-	-	NA	-	+	-	+	+	-	-	10/20 (50)
Syndactyly of toes	-	-	-	+	-	+	-	-	-	+	+	-	24/45 (53)
5th finger clinodactyly[e]	+	+	+	+	+	+	+	+	+	-	+	+	29/45 (64)
Cryptorchidism	+	+	+	+	+	-	+	-	+	female	-	-	7/42 (17)
Other									AV canal				
Patient meets at least 4 out of 8 criteria	+	+	+	+	+	+	+	+	+	+	+	+	

a) Family 1 was reported by Tekin et al.[1]; Individuals 4-1, 8-1, and 9-1 were reported as patients 6, 2, and 3, respectively in Brancati et al.[2]; b)Numbers indicate patients with a specific finding among patients with published information on that finding. Please see supplementary reference list; C) premature loss of teeth, reported on interview; d) Included in the same criterion by Skjei et al.[3]; e) indicates criteria suggested by Skjei et al.[3]; NA: Not available.

Table S2. Next-Generation Sequencing Data

Statistics	Family 2; 1	Family 1; I-1	Family 1; II-2	Average
Reads Produced	148065148	138390074	121126160	135860461
Mapped Reads	146928316	136900822	120331282	134720140
Percent of Reads that were Mapped	0.992322082	0.989238737	0.9934376	0.99166614
Reads Mapped in Pairs	146198265	134073799	117851544	132707869
Percent of Mapped Reads that were Mapped in Pairs	0.995031244	0.979349846	0.97939241	0.98459117
Proportion of Target Covered 5x or Greater	0.965258	0.9459424	0.9467005	0.95263363
Average Depth in Target	130.4887	132.7724	114.1285	125.796533

Table S3. Results of Sanger Sequencing in 11 Genes Identified in Families 1 and 2 with Exome Sequencing

Gene	Genomic Position (hg19)	Base Change	Protein Change	Family 1 (I-1 and II-2)	Genomic Position (hg19)	Base Change	Protein Change	Family 2 (ind. 1)
MICALCL NM_032867 NP_116256.2	chr11:12316030	G>A	p.Arg351Gln	Sanger confirmed/ co-segregating	chr11:12315838	A>G	p.Asn287Ser	Sanger confirmed / not de novo
CCDC88B NM_032251 NP_115627.6	chr11:64117106	C>T	p.Thr943Ile	Sanger did not confirm	chr11:64112019	G>A	p.Gly669Asp	NA
FAM86C NM_018172 NP_060642.2	chr11:71510688	A>T	p.Ser165Cys	Sanger confirmed / co-segregating	chr11:71507210	T>C	p.Tyr137His	Sanger confirmed / not de novo
GNPAT NM_014236 NP_055051.1	chr1:231403487	A>G	p.Met373Val	Sanger did not confirm	chr1:231401152	A>G	p.Lys228Glu	NA
FAM38A NM_001142864.2 NP_001136336.2	chr16:88782818	C>T	p.Ala2374Thr	Sanger confirmed/ co-segregating	chr16:88804361	G>A	p.Ala374Val	Sanger confirmed / not de novo
ABCA7 NM_019112 NP_061985.2	chr19:1053438	G>A	p.Gly1111Ser	Sanger did not confirm	chr19:1051161	G>A	p.Val898Met	NA
	chr19:1041871	C>G	p.Pro68Ala	Sanger did not confirm				
PLSCR5 NM_001085420 NP_001078889.1	chr3:146318211	G>A	p.Pro18Lys	Sanger confirmed/ not co-segregating	chr3:146311775	G>A	p.Arg129X	Sanger confirmed / not de novo
BSN NM_003458 NP_003449.2	chr3:49701910	C>T	p.Pro3888Lys	Sanger confirmed/ co-segregating	chr3:49694262	C>T	p.His2425Tyr	Sanger did not confirm
CWF19L2 NM_152434 NP_689647.2	chr11:107299915	C>A	p.Arg194Ile	Sanger confirmed/ not co-segregating	chr11:107219690	delC	p.Gly571GlefsX27	Sanger confirmed / not de novo
HERC2 NM_004667 NP_004658.3	chr15:28389352	C>T	p.Val3724Ile	Sanger confirmed/ not co-segregating	chr15:28419619	C>T	p.Val3327Met	Sanger did not confirm
ANKRD11 NM_013275.4 NP_037407.4	chr16:89341366	C>G	p.2524Glu_2525Lysdel	Sanger confirmed/ co-segregating	chr16:89350645	delA	p.Ser769GlnfsX8	Sanger confirmed / de novo

NA: Not available

Table S4. *ANKRD11* Primers Used in This Study

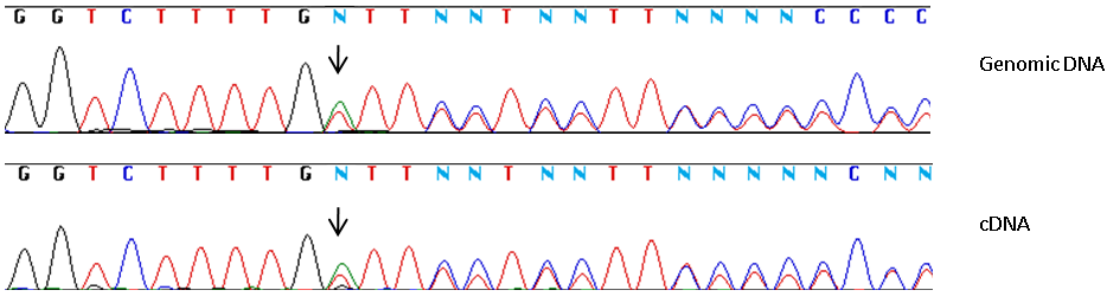
Primers	Forward Primer	Reverse Primer	PCR product size (bp)
ANKRD11 ex1	5' CGCCTCTCTCCTTCACTCC 3'	5' GCTCCGGGACCCATTATT 3'	682
ANKRD11 ex2	5' GGAGACGAGAGCGATGAAAG 3'	5' AACCTGGACACTCACGCATT 3'	245
ANKRD11 ex3	5' AGTCTGTTGAGTGCGGTGTG 3'	5' CCACCTCATCCCCATCTG 3'	285
ANKRD11 ex4	5' CAAAATCTTGAGAGCTTGAGAATG 3'	5' AGTGCAAAGCCGGAGGTG 3'	303
ANKRD11 ex5	5' GGACCTAGCTTTGGAGGACAC 3'	5' CAGCACAGCCATGAGGC 3'	311
ANKRD11 ex6	5' ATTGGACAAAGCAGGACCAC 3'	5' GAGGCTCAGGGCTCCAG 3'	318
ANKRD11 ex7	5' CTCCAGAGACAGGAGGGAAG 3'	5' ACGGGGAAACAAAGCTACAG 3'	280
ANKRD11 ex8	5' GGAACGGTAGAGGAGTTGTGC 3'	5' CACTGTGCAAACACCACAGG 3'	285
ANKRD11 ex9-1	5' CTCCCTCCTCTGCCATTCT 3'	5' TCGTGTCTTACTACCAGGCAAT 3'	500
ANKRD11 ex9-2	5' AGACACGTCGGACGAGGA 3'	5' TCTCGTCCTTGTGGAGTCTG 3'	493
ANKRD11 ex9-3	5' ACACAGACCAGCACACCAAG 3'	5' TCTCGGAAAGACCTGCTGAT 3'	589
ANKRD11 ex9-4	5' CACGATCGCGACCACTTTA 3'	5' CGGAAGAAGGGCTCTCTGTA 3'	653
ANKRD11 ex9-5	5' GAGACGGTGAAGGAGGACAG 3'	5' TGAGATGATCCCAGGGAAAG 3'	654
ANKRD11 ex9-6	5' TCTCTCGACCAAGGGAAAGA 3'	5' TCAGAGGAGACCTCGCTGAT 3'	683
ANKRD11 ex9-7	5' AGCCTGCTTGAAAAGTTGGA 3'	5' GCCTCTCCTTCTCGTCTCTC 3'	592
ANKRD11 ex9-8	5' TGGATCTAGTGCCATCAACA 3'	5' GTGCCTCAGCTTCTCCATTT 3'	496
ANKRD11 ex9-9	5' AGGACCTGGAGATCGAGGAG 3'	5' GCGGTAAAGGTTTGTGGAGA 3'	599
ANKRD11 ex9-10	5' CCCTCCTTTTTCGACAGGTT 3'	5' CTCGGAGGTGTCCAGGTC 3'	474
ANKRD11 ex9-11	5' CAGAGGGCGTCTTCTCAAGT 3'	5' TCCGGAAGTGACTTGCAGTT 3'	586
ANKRD11 ex9-12	5' AGGACGTCAAGGACGGAGT 3'	5' AGCTTCTAGAGCCACGTCCA 3'	554
ANKRD11 ex9-13	5' TCATAAACGGTGGGGATGTT 3'	5' CTGAGGGATCTCCTCCACTC 3'	485
ANKRD11 ex9-14	5' CCGAGGACGACACTGAGG 3'	5' GGACAAGACGGGCTGGAG 3'	692
ANKRD11 ex10-11	5' CCCCGTGAACTCCAGACTAT 3'	5' GAAGCTCCTGGTCAAAGTGC 3'	554
ANKRD11 ex12	5' CTTCCNGTAGCGGTCTCTG 3'	5' GTTGTCACCACCCATCACAG 3'	221
ANKRD11 ex13-1	5' CCCATACCAGGGTAGGTTCA 3'	5' ACCAGTCTGGAAGGGATGG 3'	470
ANKRD11 ex13-2	5' GGAGAGACCCCGAGAGAGAC 3'	5' AGGTCTGAAGCCCAGTCTGA 3'	420
ANKRD11 ex13-3	5' CCAGCCGTGGTTCTGTTG 3'	5' TCCTGGGAGTAAGAAGGTCGT 3'	473
Family 2 exome conf.	5' CAAACAAAGACATCAGCAGGTC 3'	5' TCGGACAAGTCAGAAAACCA 3'	370
Family 1 exome conf.	5' CTGCGTCTACAGCACAGCAT 3'	5' CTCCTGGTCAAAGTGCAGAA 3'	400
Family 2 ARMS primers (p.S769QfsX8)	5' CAAACAAAGACATCAGCAGGTC 3'	WT: 5' AATTTTGAGGGCCGGTCTTTTGA 3'	157
		MUT: 5' AATTTTGAGGGCCGGTCTTTTGT 3'	
Family 3 ARMS primers (p.Q2397X)	WT: 5' TTTCAGCGCTCCACCCAGC 3'	5' GGACAAGACGGGCTGGAG 3'	396
	MUT: 5' TTTCAGCGCTCCACCCAGT 3'		
Family 1 ARMS primers (c.7570-1G>C)	5' CTGCGTCTACAGCACAGCAT 3'	WT: 5' CAGGATACGATCAGCTTCTCC 3'	182
		MUT: 5' CAGGATACGATCAGCTTCTCG 3'	
Family 6 ARMS primers (p.P1837S)	WT: 5' CAGGGCGCCCCTGCCCC 3'	5' CTCGGAGGTGTCCAGGTC 3'	253
	MUT: 5' CAGGGCGCCCCTGCCCT 3'		
RT PCR on human brain cDNA	5'-TGGTGAACCTCCTGTTAGGC-3'	5'-CGTCGTCCTCATCAAACTCA-3'.	210

Table S5. Identified Variants in *ANKRD11* via Sanger Sequencing

Family No	Exon	Known Variants	Novel Variants					
			Nucleotide (NM_013275.4)	Protein (NP_037407.4)	Segregation	Controls	PolyPhen2	SIFT
1	10-11	-	c.7570-1G>C[a]	p.2524Glu_2525Lysdel	Co-segregating	0/339	NA	NA
2	9	-	c.2305delT[a]	p.Ser769GlnfsX8	De novo	0/255	NA	NA
3	9	rs2279348[a]	c.7189C>T[a]	p.Gln2397X	De novo	0/336	NA	NA
4	9	rs2279348[a]	c.5953_5954delCA[a]	p.Gln1985GlufsX46	De novo	0/40	NA	NA
5	9	-	c.6071_6084delCGTACGCTCTGCCC[a]	p.Pro2024ArgfsX3	De novo	0/40	NA	NA
6	9	rs2279349[a]	-	-	-	-	-	-
	9	rs2279348[a]	c.5509C>T[a]	p.Pro1837Ser	Not studied	2/300	Possibly damaging	Tolerated
7	9	rs4785560[a]	-	-	-	-	-	-
	9	rs2279349[a]	-	-	-	-	-	-
8	9	rs4785560[a]	-	-	-	-	-	-
	9	rs2279349[b]	-	-	-	-	-	-
	9	rs2279348[a]	-	-	-	-	-	-
9	9	rs2279349[b]	-	-	-	-	-	-
	9	rs2279348[a]	-	-	-	-	-	-
10	9	-	c.4683C>T[a]	p.Pro1561Ser	Unaffected mother is heterozygous	0/200	Benign	Tolerated

a)Heterozygous; b)Homozygous; NA: Not applicable

Figure S1. Sanger Sequencing of Heterozygous c.2305delT Mutation in Patient 1 of Family 2 with Genomic DNA and Complementary DNA Shows Similar Peaks



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