Supplementary Information

Results of Exome sequencing

Proband 1: The average read depth was 145X. A total of 37346 variants were obtained for the proband. The variants were filtered (see methods). A recessive mode of inheritance was firstly considered due to the known consanguinity present in the family. A total of 13 homozygous variants were observed (Table S1).

Proband 2: The average read depth was 30X. A total of 33303 variants were obtained, 12308 of which altered the protein sequence, and 880 of these were rare variants with an allelic frequency of less than 0.1%. Further variant filtering was performed using in-house allelic frequency data, resulting in the total of 309 variants. Homozygous and compound heterozygous variants were firstly evaluated, with the presence of a total of seven homozygous variants (Table S2).

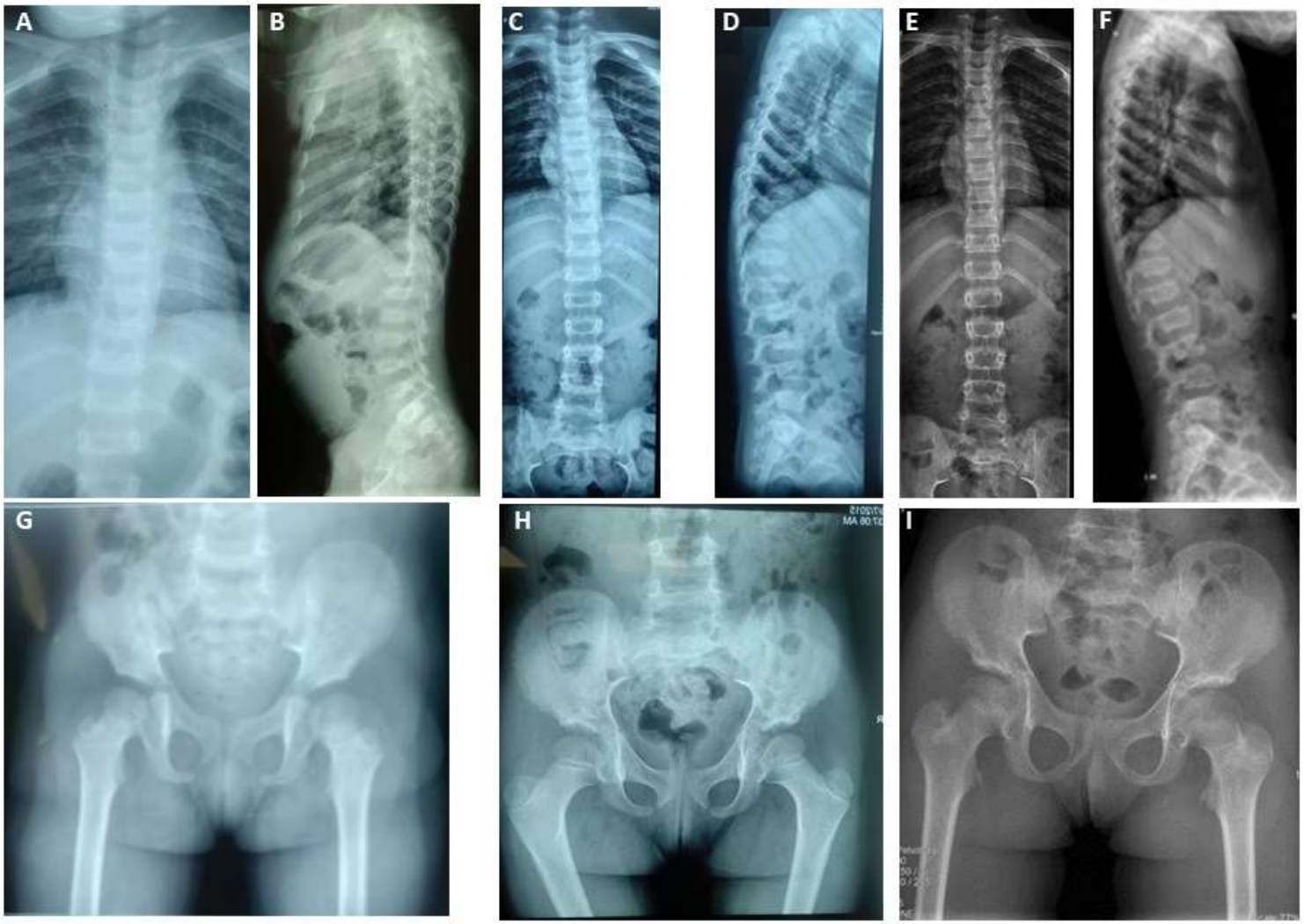
Díaz-González F, *et al. J Med Genet* 2021; 59:28–38. doi: 10.1136/jmedgenet-2020-107177

Supplemental Figure legends

Figure S1: Growth curve of the female proband from family 1. Height and weight data are indicated by black spots, bone age (red spot), adult target height (green spot and post-lengthening height (yellow spot). At age 12, she had advanced bone age (3 years) in relation to her chronological age. The growth curve is based on 2010 Spanish height data (http://www.aeped.es/noticias/estudios-espanoles-crecimiento-2010). The coloured lines represent the growth percentiles.

Figure S2: Evolution of skeletal anomalies of proband 2 at age 4, 7 and 11 years old. Radiographs at 4 years (A, B, G, J, L, O), 7 years (C, D, H, M, P) and 11 years (E, F, I, K, N, Q). At age 4, moderate platyspondyly with anterior beaking of vertebral borders of dorso-lumbar spine was observed (A, B) which improved with age, at 7 years (C, D), and at 11 years (E, F). The ilia were short with flaring of the iliac wings (G, H, I at 4, 7, and 11 years old, respectively). At 7 years old, she had relatively large epiphyses and increased prominence of metaphyseal irregularity of long bones (P), which was not observed at age 4 (O) and that improved by age 11 (Q). Prominent deltoid tuberosities of the humeri were observed (J, K, at age 4 and 7 years respectively). She had short and broad phalanges and metaphyseal irregularity of metacarpals (L, M, N, at 4, 7, and 11 years old, respectively) and metatarsals (data not shown).

Díaz-González F, *et al. J Med Genet* 2021; 59:28–38. doi: 10.1136/jmedgenet-2020-107177


A
B
C
D
E
F
G
H
I

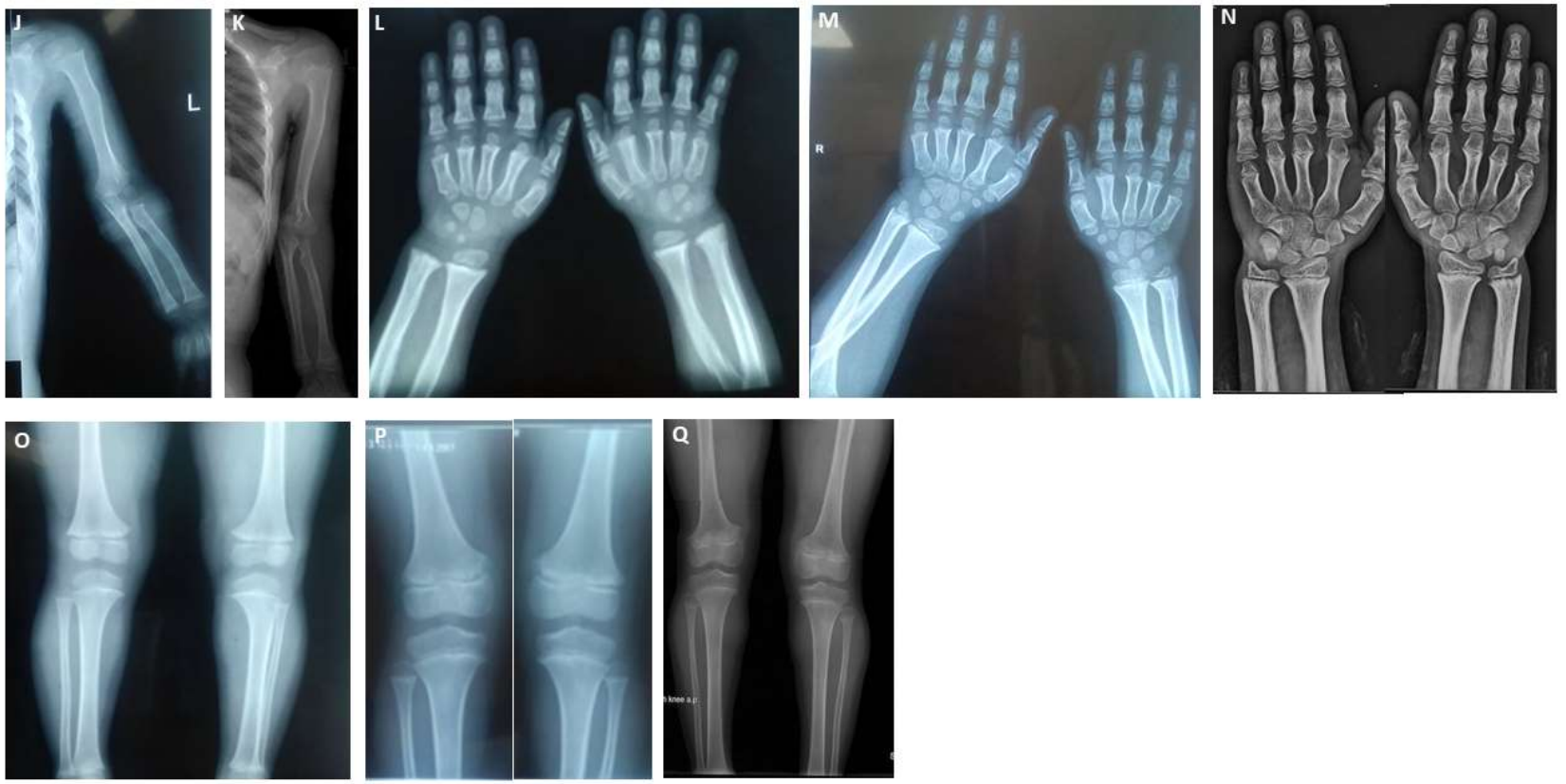
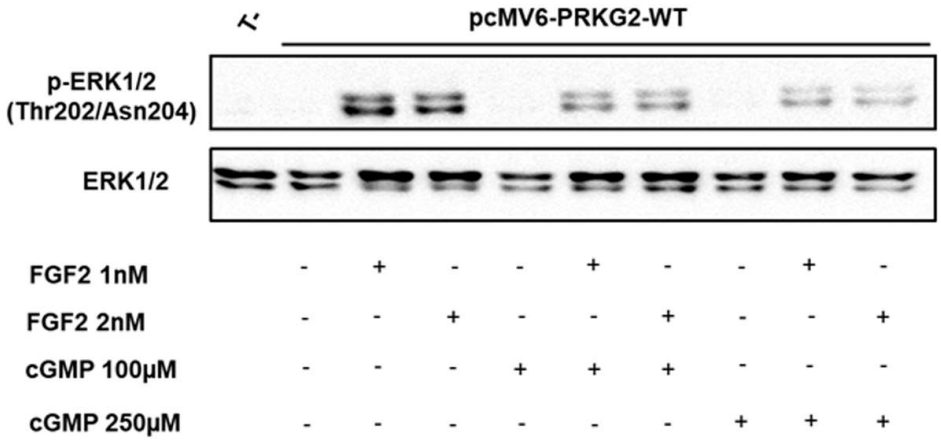

J
L
K
L
M
R
N
O
P
Q

Fig S3: cGMP reduces FGF2 induced activation of p-ERK1/2 in HEK293T cells transfected with PRKG2-WT. Cells were treated with 100μM or 250μM cGMP for 30 minutes followed by the addition of 1nM or 2nM FGF2 for another 30 minutes. Cells were collected and lysed to obtain the protein. Western blot was subsequently performed for the determination of the activation/inhibition of ERK1/2 pathway as described in methods. FGF2 treatment activates ERK1/2 phosphorylation and the addition of cGMP dramatically reduces p-ERK1/2 independently of the concentration used.



Supplemental Tables

Table S1: Homozygous variants detected in the exome sequencing of proband 1.

Chr.	Position (hg19)	Ref/Alt	Allelic depth	Gene	Variant cDNA and protein	Disorder [MIM]	Mode of inheritance
1	32193790	G/A	0/189	*ADGRB2*	NM_001294335.1:c.4505C>T; p.Thr1502Met	-	-
1	33233498	C/T	1/231	*KIAA1522*	NM_020888.2:c.346C>T; p.Pro116Ser	-	-
1	45166717	G/C	0/94	*C1orf228*	NM_001145636.1:c.565G>C; p.Gly189Arg	-	-
1	55172208	T/C	0/107	*MROH7*	NM_001039464.3:c.3665T>C; p.Ile1222Thr	Limb girdle muscular dystrophy [MIM 608807]	AR
2	179393898	T/A	0/48	*TTN*	NM_001267550.2:c.106580A>T; p.Glu35527Val	Salih myopathy [MIM 611705]	AR
2	179398515	C/T	1/87	*TTN*	NM_001267550.2:c.102827G>A; p.Arg34276Gln	Salih myopathy [MIM 611705]	AR
2	179410721	G/A	0/199	*TTN*	NM_001267550.2:c.95242C>T; p.Arg31748Cys	Salih myopathy [MIM 611705]	AR
2	179448450	G/A	0/103	*TTN*	NM_001267550.2:c.65459C>T; p.Thr21820Ile	Salih myopathy [MIM 611705]	AR
2	179466171	T/C	0/132	*TTN*	NM_001267550.2:c.55553A>G; p.Lys18518Arg	Salih myopathy [MIM 611705]	AR
2	179486345	T/A	0/135	*TTN*	NM_001267550.2:c.45206A>T; p.Glu15069Val	Salih myopathy [MIM 611705]	AR
4	**82056380**	**G/A**	**0/102**	***PRKG2***	**NM_006259.2:c.1705C>T; p.Arg569***	**-**	**-**
4	84390212	C/A	0/40	*FAM175A (ABRAXAS1)*	NM_139076.2:c.569G>T; p.Gly190Val	-	-
11	16824599	C/T	0/43	*PLEKHA7*	NM_001329630.1:c.2077G>A; p.Val693Ile	-	-

The pathogenic *PRKG2* variant is highlighted in bold. Chr. Chromosome; Ref: reference, Alt: variant; AR: autosomal recessive.

Table S2: **Homozygous variants detected in the exome sequencing of proband 2.**

Chr.	Position (hg19)	Ref/Alt	Allelic depth	Gene	Variant cDNA and protein	Disorder [MIM]	Mode of inheritance
4	69098125	T/C	1/15	*TMPRSS11B*	NM_182502:c.479A>G; p.Lys160Arg	-	-
4	**82096083**	**A/AT**	**0/14**	***PRKG2***	**NM_006259:c.491dupA; p.Asn164Lysfs*2**	**-**	**-**
4	1.06E+08	A/G	0/6	*TET2*	NM_001127208:c.3583A>G; p.Ile1195Val	Myelodysplastic syndrome, somatic [MIM 614286]	Somatic
4	1.4E+08	T/A	0/8	*SETD7*	NM_001306199:c.898A>T; p.Thr300Ser	-	-
4	1.48E+08	C/T	0/9	*POU4F2*	NM_004575:c.1028C>T; p.Ala343Val	-	-
22	50664251	C/T	0/13	*TUBGCP6*	NM_020461:c.1955G>A; p.Arg652His	Microcephaly and chorioretinopathy, autosomal recessive, 1 [MIM 251270]	AR
22	50905771	G/A	0/17	*SBF1*	NM_002972:c.545C>T; p.Ser182Leu	Charcot-Marie-Tooth disease, type 4B3 [MIM 615284]	AR

All variants are Quality filtered passed and Phred-scaled likelihoods (PL) values of 0 for the called genotype. The pathogenic *PRKG2* variant is highlighted in bold. Chr. Chromosome; Ref: reference, Alt: variant; AR: autosomal recessive.