Biallelic Gene Disrupting Variants in *PKDCC* Cause a Skeletal Disorder Characterized by Rhizomelic Shortening of Extremities and Distinctive Facial Features

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BACKGROUND

- The skeletal dysplasias are heterogeneous disorders of cartilage and bone that usually cause short stature (and sometimes overgrowth) with an incidence of 1/5000 live births[1]. There are about 400 individual skeletal dysplasia disorders currently recognized and 300 causal genes[2].
- The *Protein kinase domain containing, cytoplasmic* gene, also known as *Pkdcc* or *Vlk,* is expressed in the ventral part of the mid-brain, with strong signals seen in branchial arches and limb buds during mouse development[3,4].
- Homozygous *Pkdcc* knockout mice have shortened long bones due to delayed endochondral ossification as well as craniofacial abnormalities including small and shortened nasal capsule and maxilla[3,4].
- We present the first report of two unrelated patients with homozygous gene disrupting variants in *PKDCC* who had rhizomelic shortening of limbs and distinctive facial features

Table 1. Clinical features of patients reported in this study

	Patient 1	Patient 2
Current age (and gender)	17-years-old (Female)	2-years-old (Male)
Consanguinity	None	None
Birth measurements	Weight-3.18kg (32nd centile); Length-50.8cm (69th centile)	Unknown
Most recent measurements	At 17 years: Weight-73.6kg (92nd centile); Height-156.7cm (17th centile); Head circumference-55.4cm (84th centile); BMI-30 (96th centile)	At 19 months: Weight-10.3kg (8th centile); Height-77.3cm (5th centile); Head circumference-52.5cm (>98th centile); BMI-30 (96th centile)
Age when diagnosed	2-months-old	4-months-old
Skeletal findings on radiographs	Rhizomelic shortening and milder mesomelic shortening of the upper and lower extremities, short thumbs, bilateral short 5th fingers, hyperextensible fingers, bilateral middle finger clinodactyly, limited range of motion of shoulder joints, chronic joint pain, juvenile idiopathic arthritis, and bilateral patellofemoral joint dislocation.	Rhizomelic short stature most evident in upper extremity, prominent fingertip pads and a simian crease on the left, flattening of dorsal aspect of the skull indicative of plagiocephaly but otherwise grossly normal
Craniofacial features	Prominent forehead, downslanting palpebral fissures, broad nasal bridge, long philtrum	Macrocephaly, short neck, micrognathia, mild proptosis, depressed nasal bridge, long smooth philtrum.
Other findings	Obesity, left sided headaches, acanthosis nigricans, chronic stage 1 kidney disease, a café au lait macule on upper right arm, depressed mood, occasional abdominal pain, dizziness, nausea, and a left sided small branchial cleft defect covered with skin without an obvious fistula.	Difficulty gaining weight, receives physical and speech therapies, central hypotonia, bilateral mild conductive hearing loss, laryngomalacia, poplyploid anal mass which had decreased in size, brisk deep tendon reflexes
Brain and spine MRI	Unknown	At 9 months showed mildly delayed myelination and benign enlargement of subarachnoid spaces of infancy without aqueductal stenosis. No craniocervical junction anomalies in the cervical region of the spine.
Cardiac	Unknown	Heart murmur, mildly increased velocity in the suprapulmonic region with no pulmonary valve stenosis, a small patent foramen ovale with left to right shunting, and mild tricuspid regurgitation.

METHODS

- Trio-based diagnostic whole exome sequencing was performed on the proband and parents as previously described[5] with the exception of the capture reagent used which was IDT xGen Exome Research Panel V1.0. Approximately 97% of each individual's exome was covered at 20x or higher.
- Population frequency cutoffs for variant filtering included 0.1% for dominant, 1% and 0.2% for recessive characterized and uncharacterized genes, respectively, and 0.2% for incomplete penetrance models. The latter model included variants that are found in the Human Gene Mutation Database (HGMD), those that cause truncations such as frameshift, nonsense, and canonical splice, and those found in known imprinted disorder-causing genes. Population databases used were 1000 Genomes Project, Exome Sequencing Project (ESP), and Exome and Genome Aggregation Consortia (ExAC and gnomAD, respectively). Candidate gene analysis was done as previously described[6].

RESULTS

Table 2. Post-filtering summary of variants in each patient

	Patient 1	Patient 2
Number of variants (and genes) post-filtering remaining in characterized disease genes -- all were considered clinically irrelevant to both patients' phenotypes	10 (8)	4 (4)
Number of variants (and genes) post-filtering remaining in uncharacterized disease genes	3 (2)	1 (1)
Variants in uncharacterized genes considered to be candidate findings that explained the patients' phenotype	PKDCC NM_138370 c.651C>A p.Y217*	PKDCC NM_138370 c.639+1G>T
Genotype	Homozygous (each parent heterozygous)	Homozygous (each parent heterozygous)
dbSNP ID of candidate variants	rs761532715	rs763243200
Location of variant in candidate gene	Exon 2	Intron 1
Nature of variant in candidate gene	Nonsense	Canonical donor splice
Expected effect on mRNA	Non-sense mediated decay resulting in complete loss of protein	Abolishment of the canonical donor splice based on three in-silico prediction algorithms (Berkeley Drosophila Genome Project, ESE Finder, and Human Splice Finder)
Highest sub-population allele frequency in the gnomAD control database	0.02735% (6/21936 Finnish alleles) with 0 homozygotes	0.02746% (6/21850 Latino alleles) with 0 homozygotes

Figure 1. Integrated Genome Browser (IGV) view showing the homozygous PKDCC candidate variants identified in Patient 1 (left) and Patient 2 (right). Each parent of both patients was heterozygous. The number of reads containing the variant allele and reference allele in each individual are also shown.

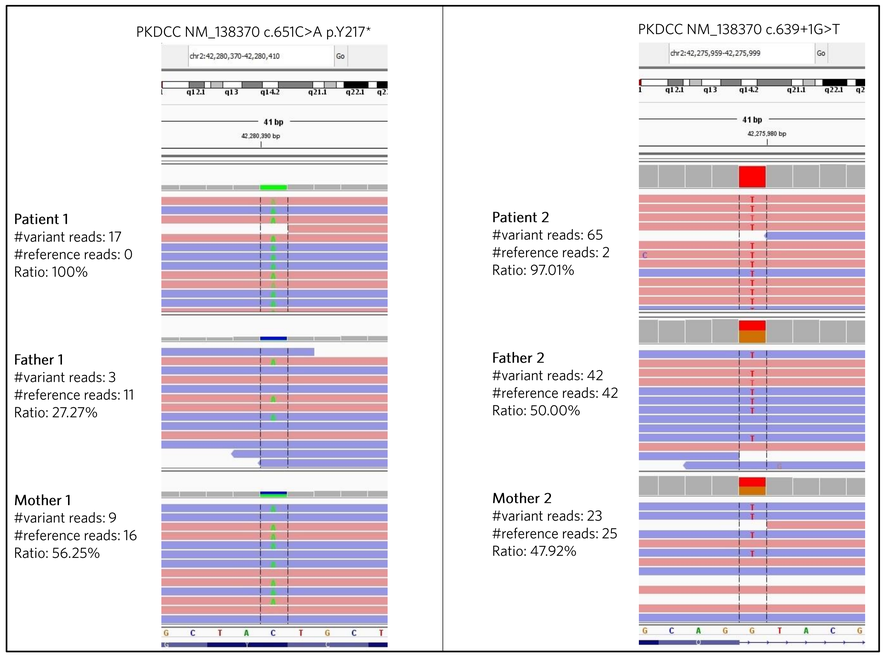
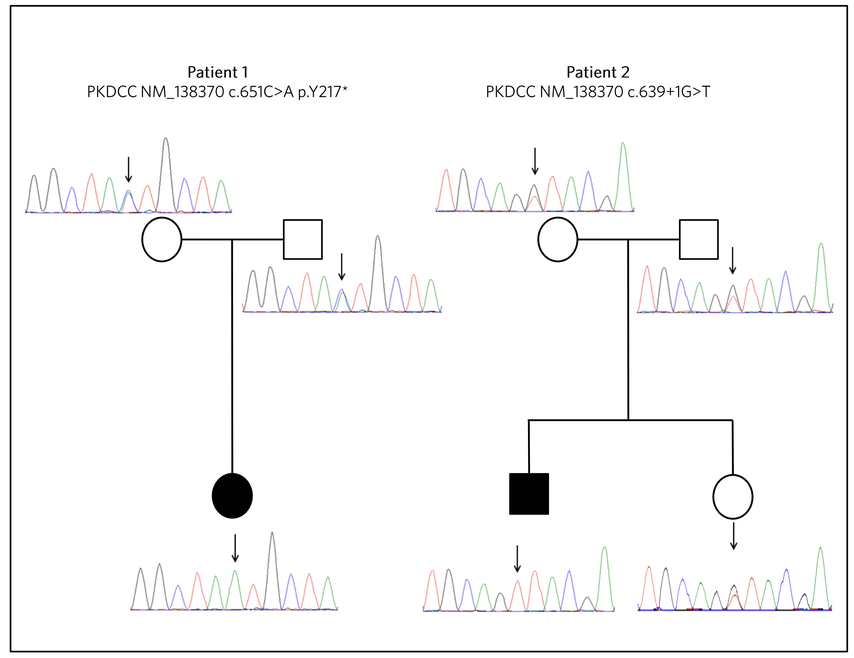


Figure 2. Pedigrees and Sanger confirmation of the homozygous *PKDCC* candidate variants shown by arrows in Patient 1 (left) and Patient 2 (right).



INTERPRETATION

- The clinical and genetic data of the two patients presented here show that biallelic gene disrupting variants in *PKDCC* can cause skeletal defects and distinctive facial features, making this a new candidate gene for skeletal dysplasia in humans. Additional lines of evidence that support this assertion include:

1. In mice, Pkdcc affects bone development through hedgehog (HH) signaling by interacting with Gli3 and Ihh proteins[7]. Homozygous knockout mice for genes that encode the latter two proteins also display skeletal abnormalities comprising of short limbs and craniofacial abnormalities[8,9].
2. Double homozygous knockout mice for *Pkdcc* and *Gli3* display skeletal defects that are more severe than single knockout mice, showing that Pkdcc also acts through the HH pathway to affect bone development[6].
3. In humans, mutations in both *GLI3* and *IHH* genes are known to cause skeletal disorders (OMIM #165240 and OMIM #600726, respectively), both of which involve short stature, shortening of digits and/or limbs as well as some dysmorphic features.
4. As mentioned above, during mouse embryonic development, the *Pkdcc* gene is strongly expressed in the branchial arches and limb buds, with homozygous *Pkdcc* knockout mice displaying short stature and craniofacial abnormalities[3,4].

TAKE-HOME POINTS

- Via trio-based diagnostic exome sequencing (DES) we have identified a novel human disease gene, *PKDCC*, that causes skeletal abnormalities in humans due to biallelic loss of function variants.
- The phenotype of the two human patients resembled that of knockout mice, thereby providing support for the role of this gene in human skeletal development.
- DES is a useful tool for identifying novel human disease genes.

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