Whole-exome sequencing as a powerful tool to unravel the molecular pathogenesis of osteogenesis imperfecta

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Osteogenesis imperfecta (OI) comprises a heterogeneous group of disorders characterized by bone deformities, low bone mass, brittle bones, and connective tissue manifestations. Dominant pathogenic variants in the *COL1A1* or *COL1A2* genes account for more than 80% of the cases, whereas recessive defects can be found in a plethora of genes. In a small subset of patients, the genetic basis remains elusive, suggesting the involvement of other, yet to be discovered, genes. The aim of our study is to identify the causal defect in molecularly unexplained OI patients.

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Hitherto, in two patients pathogenic variants were identified in the *CTSK* and *PIEZO2* genes, while in two other patients variants of unknown significance were identified in genes known to be associated with the OI phenotype.

Our findings illustrate that WES is not only a valuable tool for the detection of pathogenic variants in known OI genes, but also in genes associated with phenotypically related disorders. This study illustrates that the use of WES-based methods is a powerful tool in genetically elucidating phenotypical overlapping disorders.

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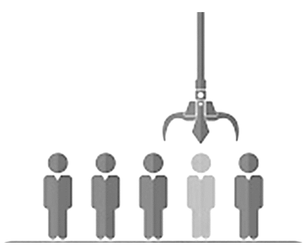

Nineteen *COL1A1*/*COL1A2* negative OI-like cases were selected for whole-exome sequencing (WES), based on clinical severity and positive familial anamnesis.

Sampleprep was performed using the Agilent SureSelect XT target enrichment system. After pooling and quantification by means of qPCR, libraries were sequenced on the Nextseq500 or HiSeq3000 systems (Illumina).

Mapping and data analysis were performed using commercial (CLC workbench) and in house developed tools.

Variant prioritization was based on literature and the Ingenuity Variant Analysis software. Sanger sequencing was used for variant confirmation and segregation analysis.

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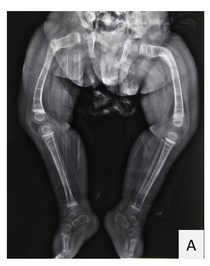
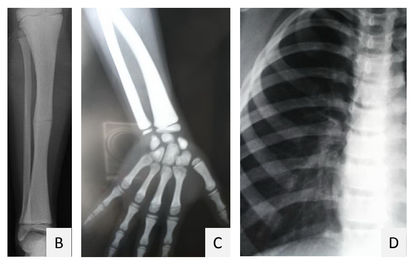
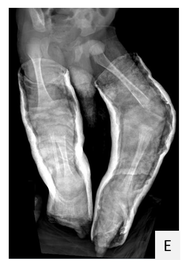
	P1	P2	P3	P4
Sex / Age at diagnosis	male / 2y	female / 16y	male / 1y	female / 1y
				
Clinical phenotype				
	First fracture of femora at day 2 of life, 6 fractures in total in first 2 years (which heal with deformities), short stature, no blue sclerae, triangular face, brachiocephaly, hyperelasticity, flat occiput, psychomotor retardation (see image A).	Multiple fractures (first fracture at 4 years of age), osteopetrosis, mild short stature, long palpebral fissure, pinched nose with long columella, blue sclerae, dentinogenesis imperfecta (see images B-C-D).	Oligoamnios, narrow palpebral fissure, microretrognathia, low set ears, contractures of elbow and knees, muscle atrophy of lower limbs, lower femoral epiphysis / absent upper tibial epiphysis, long fingers without creases, talipes, bilaterally prefixed halluces (see image E).	No fractures, short stature, blue sclerae, dimples, bowed humeri.
Genotype/function				
Gene	***LEPRE1 (P3H1)***	***CTSK***	***PIEZO2***	***ALPL***
Protein function	Prolyl-3-hydroxylase-1 is required for proper collagen biosynthesis, folding, and assembly.	Cathepsin K is a cysteine protease which digests bone matrix proteins during bone resorption.	Transmembrane cation channel which plays a role in adapting mechanically-activated currents in somatosensory neurons.	Liver / bone / kidney alkaline phosphatases are membrane-bound glycoproteins, playing a role in bone mineralization.
Inheritance	AR	AR	AD	AR
cDNA change	c.446[T>G];[T>G]	c.721[C>T];[C>T]	c.8057[G>A];[=]	c.1444[C>A];[C>A]
Protein change	p.[(Leu149Arg)];[(Leu149Arg)]	p.[(Arg241*)];[(Arg241*)]	p.(Arg2686His)	p.[(His482Asn)];[(His482Asn)]
Variant information				
Population frequency (gnomad)	unknown	6.904e-5 (0 homozygotes)	unknown	1.111e-5 (0 homozygotes)
PhyloP/PhastC	3.676/1	0.286/0.244	5.613/1	3.272/1
Rs	-	rs7431530	-	rs780857373
Sift/PolyPhen	deleterious/probably damaging	-/-	deleterious/probably damaging	deleterious/probably damaging
AGVD/Grantham score	C0/102	-/-	C0/29	C0/68
Literature	-	Known pathogenic variant[1]	Known pathogenic variant[2]	Causality unclear[3]
Variant classification (ACMG)	CLASS 3	CLASS 5	CLASS 4	CLASS 3

[1] Johnson MR et al, Genome Res. 6: 1050-1055, 1996: A nonsense mutation in the cathepsin K gene observed in a family with pycnodysostosis.
[2] McMillin MJ et al, Am J Hum Genet. 94(5): 734-44, 2014: Mutations in PIEZO2 cause Gordon syndrome, Marden-Walker syndrome, and distal arthrogryposis type 5.
[3] Nielson CM et al, J Bone Miner Res. 27(1), 2012: Rare Coding Variants in ALPL Are Associated With Low Serum Alkaline Phosphatase and Low Bone Mineral Density.