Implementation of an in-house designed skeletal dysplasia gene panel as a first screening step to diagnose unsolved osteogenesis imperfecta (–like) patients

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The pace of disease-related gene discovery has accelerated phenomenally in recent years due to advances of next generation sequencing (NGS) technologies, such as whole exome sequencing (WES). In the field of skeletal dysplasias, particularly for osteogenesis imperfecta (OI), NGS added a remarkable number of disease loci, thereby expanding the brittle bone disease to a predominantly collagen-related disorder. However, for a small subset of OI patients the genetic cause remains elusive. We designed a skeletal dysplasia gene panel and evaluated the detection rate in a cohort of unsolved OI (-like) patients.

Applications

Exome sequencing can efficiently identify coding variants across a wide range of applications, including population genetics, genetic disease, and cancer studies

Targeted sequencing

WES is perhaps the most widely used targeted sequencing method

Exome vs genome

The exome, the protein-coding region of the human genome, accounts for less than 2% of the genome but contains ~85% of known disease-related variants

Success rate

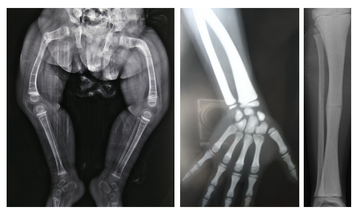
In clinical setting, reported detection rates for deleterious mutations range from 25 to 50%

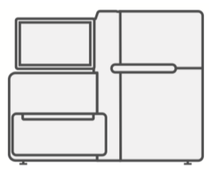
Searching for the needle in the haystack

An average of 41636 variants were generated using the GRCh38/hg38 build across the 21 exome samples included in this study

Figure 1: Whole exome sequencing, facts and figures.[1,2]

Twenty-one OI-like patients, in who no type I collagen defect was detected, were selected based on clinical severity (most patients had multiple fractures) and positive familial anamnesis (patients with a consanguineous background were prioritized during the selection procedure). WES sample prep was performed using the Agilent SureSelect XT target enrichment system.





WES libraries were sequenced on Illumina platforms, data were analysed by SeqPlorer (an in-house developed pipeline, which integrates population database information/quality scores/coverage data and *in silico* prediction algorithms).

The generated data was mapped against a newly designed skeletal dysplasia gene panel, including 566 genes. This gene panel includes known loci for heritable skeletal disorders, but also candidate genes for bone fragility are included (which are the result of GWAS and animal studies linking aberrant BMD scores to candidate genes for osteoporosis).[3,4,5,6,7]

Figure 2: Methods used in this study.

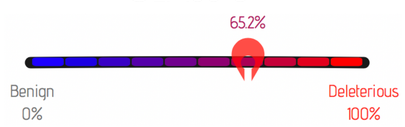
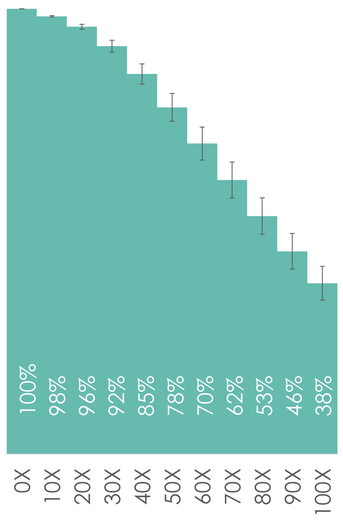
	***LEPRE1* – case (male, 2y)**	***ALPL* – case (female, 1y)**	***CTSK* – case (female, 16y)**
Clinical phenotype	Six fractures in first 2 years, short stature, triangular face, brachiocephaly, flat occiput, psychomotor retardation	No fractures, short stature, blue sclerae, dimples, bowed humeri	Multiple fractures, osteopetrosis, mild short stature, long palpebral fissure, pinched nose with long columella, blue sclerae, dentinogenesis imperfecta
Protein function	Prolyl-3-hydroxylase-1 (P3H1) is required for proper collagen biosynthesis, folding, and assembly	Liver / bone / kidney alkaline phosphatases are membrane-bound glycoproteins, playing a role in bone mineralization.	Cathepsin K is a cysteine protease which digests bone matrix proteins during bone resorption.
Protein change	p.[(Leu149Arg)];[(Leu149Arg)]	p.[(His482Asn)];[(His482Asn)]	p.[(Arg241*)];[(Arg241*)]
Clinical diagnosis	autosomal recessive (AR) OI	AR hypophosphatasia	AR osteopetrosis
Variant classification	CLASS 3	CLASS 3	CLASS 5
DEOGEN2 [8,9]			DEOGEN2 is not applicable for nonsense variants

Figure 3: Overview of the 3 solved cases.

Figure 4: Average skeletal dysplasia gene panel coverage of the 21 exomes included in this study. An exome is considered as 'well sequenced' if more than 95% of the bases is 20x covered. Error bars, SD.



Conclusions and future perspectives

Our findings show that the implementation of this skeletal dysplasia gene panel is a powerful first screening step in molecularly diagnosing OI(-like) patients, especially for those patients whom are difficult to diagnose due to phenotypically overlapping clinical symptoms.

With regards to the 18 unsolved exome cases: with the use of SeqPlorer, we were able to generate a shortlist of 71 variants in total, of which further prioritization, confirmational sanger sequencing, segregational analysis and functional studies will have to aid in the elucidation of these cases. Besides the used seqplorer pipeline, we are currently testing tools in order to pick up copy number variations from the same exome datasets (such as cn.MOPS, CoNIFER and ExomeDepth).

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