**Citation:** BoneKEy Reports **3**, Article number: 528 (2014) | doi:10.1038/bonekey.2014.23

**www.nature.com/bonekey**

## COMMENTARY

# Lessons from next-generation sequencing in genetic skeletal disorders

**Maria L Brandi**

Department of Surgery and Translational Medicine, University of Florence, Florence, Italy.

*BoneKEy Reports* **3**, Article number: 528 (2014) | doi:10.1038/bonekey.2014.23; published online 14 May 2014

**Commentary on:** Sule G, Campeau PM, Zhang VW, Nagamani SC, Dawson BC, Grover M, Bacino CA, Sutton VR, Brunetti-Pierri N, Lu JT, Lemire E, Gibbs RA, Cohn DH, Cui H, Wong LJ, Lee BH. Next-generation sequencing for disorders of low and high bone mineral density. *Osteoporos Int* 2013;**24**(8):2253–2259.

The Human Genome Project sought to determine the order of all 3 billion nucleotides in the human genome, and this was made possible through the development of sequencing techniques that emphasized speed with attention also to accuracy. From the early labor-intensive efforts at sequencing genes, the situation became to change during the mid-1970s thanks to the groundbreaking discoveries of Nobel Laureate Frederick Sanger.[1] The Sanger sequencing process, also called the chain-termination or dideoxy method, was characterized by the introduction of chain-terminating dideoxynucleotides, making the procedure sensitive enough to distinguish DNA fragments that differ in size by only a single nucleotide. In the 1980s, automated DNA sequencing machines were manufactured based on the Sanger method. Although very expensive, these machines provided sequence data cheaper and faster than the traditional method. Indeed, these technologies put Craig Venter in the position of a quick completion of the Human Genome Project.

Over time, sequencing technology (and synthesis technology) advanced with more sophisticated separation strategies, alternative visualization strategies and more parallel samples, collectively indicated as next-generation sequencing (NGS), first described in 2005.[2] NGS platforms provide massively parallel sequencing of millions of DNA fragments via synthesis, drastically cutting the cost of sequencing and eventually allowing every person the possibility of personalized genome information.[3] NGS methods are not limited to sequencing genomic DNA, but also RNA, the epigenome and transcriptome.[4] However, it may be not necessary to sequence an individual's entire genome, but only an obvious subset of the genome likely harboring significant mutations, that is about the one percent that encodes for protein, known as exome.[5] Whole-exome sequencing (WES) seems particularly suited for gene identification in rare Mendelian disorders. Being able to read our genes and their sequence functionality offers the promise of advanced medical treatments, but it will require considerable efforts to generate, organize and apply this massive amount of data to human diseases.

The application of NGS to genetic disorders has revolutionized the ability to rapidly develop molecular diagnosis in inherited diseases, especially monogenic disorders.[6] Furthermore, the cost of NGS is rapidly decreasing and has made tangible the prospect of incorporating genome-based diagnosis into medical care.[7] In this Commentary, we focus on the application of these developments to congenital bone disorders.

Skeletal dysplasias are congenital disorders that affect skeletal morphogenesis and metabolism, usually monogenic, with obvious Mendelian inheritance within families. The most recent classification of these disorders was published in 2010 and it was based on clinical and radiologic features, with the final recognition of 456 different conditions and a collective incidence of 1:5000 births.[8,9] Of these conditions, 316 were associated with 226 different genes mainly recognized through parametric linkage studies.[8] This typically requires large pedigrees with informative meiosis, a condition frequently missing in families with skeletal dysplasias for often compromised fertility and life expectancy. Consequently, the molecular cause of several congenital rare bone disorders remains unrecognized.

Moreover, what makes this clinical area unique is the difficult differential diagnosis among similar phenotypes, with complex screening for the definition of causative mutations even when the responsible genes have been already identified. The clinical diagnosis of skeletal dysplasias is mainly based on radiographic and metabolic profiles, whose overlapping phenotypes are considerable. The consequence of this is the complexity in reaching a molecular diagnosis in genetically different skeletal dysplasias with a similar clinical phenotype, meaning many genes may require sequencing.

Altogether, these observations point to the potential use of NGS platforms in accelerating the genetic diagnosis of skeletal dysplasias and in identifying novel causative genes for this family of disorders. Very recently, a few reports appeared focusing on the use of NGS in skeletal dysplasia, and specific examples of these approaches are given in this Commentary.

To date, 36 reports described the use of NGS to identify 28 novel causative genes for skeletal dysplasias, pointing to the importance of these methodologies in promoting the progresses in this important area of medicine.[10] A good

example is the discovery of a single-point mutation of the bone-restricted *Ifitm-like* gene (*Bril*) as the causative mutation in osteogenesis imperfecta type V (OI type V).[11] Similarly, in Marfan syndrome, an autosomal dominant hereditary connective tissue disorder, NGS provided us new insights into the molecular events governing the pathogenesis of this disease.[12,13]

OI is an example of heterogeneity in heritable disorders of bone fragility, and for this reason it has been the subject of great interest from researchers. The condition is usually diagnosed clinically, as genetic testing is expensive owing to the size and number of potentially causative genes and mutations. As the genetic diagnosis has positive impact on prognosis, on targeted-therapeutic strategies, on reproductive planning and on prenatal recognition, the need to develop technologies that would ease the genetic recognition of this disorder is urgently felt. An attempt to guide the process was described using capillary electrophoresis-based Sanger sequencing of multiple genes in a sequential manner, which is, however, costly and time-consuming.[14] Through NGS it would be possible to build platforms specifically constructed for OI genes.[15] These methodologies could also unravel the mechanisms that explain the phenotypic differences in subjects with the same mutation, through the evaluation of the individual's epigenetic profiles.

Interestingly, a group of investigators designed a NGS platform to sequence 34 genes that provided a fast and accurate diagnosis in 11 subjects with inherited high or low bone mineral density.[16] This work made it possible to include rare skeletal dysplasias among clinically available disease-targeted tests,[17] as NGS offers novel solutions in reaching a genetic diagnosis, with the possibility to use other molecular diagnostic approaches when a mutation cannot be identified (that is, microdroplet PCR, multiplex ligation-dependent probe amplification, array comparative genomic hybridization, exome sequencing, gene expression and biochemical studies).[16] The choice of the technology to be adopted should take into consideration the cost, sensitivity and specificity in a dynamically progressing process. Clinical implementation of the skeletal dysplasia NGS-targeted platform has the potential to diagnose patients with monogenic bone disorders with a high degree of speed and accuracy, offering a more focused picture of monogenic and polygenic contributors to bone dysplasias, without providing information about incident pathogenesis.

The paper by Sule *et al.*[16] opens an avenue for the genetic diagnosis of a large number of diseases involving the skeleton, through a categorization based on bone mineral density measurement. Even though this can be considered a potential approach, bone mineral density does not constitute a recognized pathophysiological way to group congenital skeletal disorders. Although the majority of the efforts made to classify these disorders was based on radiologic features,[8] a reasonable manner to categorize congenital skeletal diseases could encompass their metabolic phenotype. In this direction is moving the Rare Skeletal Diseases Working Group of the International Osteoporosis Foundation that is attempting to build a taxonomy of Rare Congenital Metabolic Bone Disorders. As remodeling is the way through which the shape, the bone mineral density and the architecture of bone segments are controlled, this could represent an excellent template on which to build an NGS platform for skeletal dysplasia.

In the study by Sule *et al.*,[16] the authors point to the usefulness of postnatal genetic tests in low and high bone mass disorders to help initiate appropriate therapeutic interventions. An example is OI for which the use of bisphosphonates is largely diffuse. Another emerging area is the one of hypomineralization, typically represented by the rare inherited metabolic bone disorder hypophosphatasia. Diagnosis of hypophosphatasia can be suggested by clinical and radiographic signs of bone and/or teeth defective mineralization. As these signs are not specific, laboratory assays are necessary for the differential diagnosis with other bone fragility conditions characterized by defective mineralization.[18] Sequencing of the gene encoding for the tissue-nonspecific alkaline phosphatase becomes necessary to confirm the diagnosis of hypophosphatasia, with over 200 different disease-causing mutations having been reported.[19] This will make possible the access to a recently developed enzyme replacement-targeted therapy.[20]

Even though the expansion of new technologies in genetic tests is increasing the likelihood of a diagnosis, the question remains whether these tests are really making a difference in patient care and at what cost. This is the reason for which different testing strategies have not yet been extended to rare diseases, given the limited data available. Future efforts should be made to validate these novel molecular diagnostic efforts in large cohorts of phenotypically well-characterized populations of patients affected by congenital bone disorders. Despite challenges, NGS is going to rapidly move toward use in a clinical capacity, and rare congenital bone disorders will become an important application area.

## Conflict of Interest

I declare that I do not have conflicts of interest with the content of this paper, as I am consultant to and grants recipient from Amgen, Eli Lilly, MSD, Novartis, Roche and Servier.

## References


1. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977;**74**:5463–5467.
2. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA *et al.* Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 2005;**437**:376–380.
3. Zhang J, Chiodini R, Badr A, Zhang G. The impact of next-generation sequencing on genomics. *J Genet Genomics* 2011;**38**:95–109.
4. Saletore Y, Meyer K, Korlach J, Vilfan ID, Jaffrey S, Mason CE. The birth of the epitranscriptome: deciphering the function of RNA modifications. *Genome Biol* 2012;**13**: 175–186.
5. Gilissen C, Hoischen A, Brunner HG, Veltman JA. Unlocking Mendelian disease using exome sequencing. *Genome Biol* 2011;**12**:228–238.
6. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA *et al.* Clinical whole-exome sequencing for the diagnosis of Mendelian disorders. *N Engl J Med* 2013;**369**: 1502–1511.
7. Katsanis SH, Katsanis N. Molecular genetic testing and the future of clinical genomics. *Nat Rev Genet* 2013;**14**:415–426.
8. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M *et al.* Nosology and classification of genetic skeletal disorders—2010 revision. *Am J Med Genet* 2011;**155**:943–968.
9. Krakow D, Rimoin DL. The skeletal dysplasias. *Genet Med* 2010;**12**:327–341.
10. Lazarus S, Zankl A, Duncan EL. Next-generation sequencing: a frameshift in skeletal dysplasia gene discovery. *Osteoporos Int* 2014;**25**:407–422.
11. Lazarus S, Moffatt P, Duncan EL, Thomas GP. A brilliant breakthrough in OI type V. *Osteoporos Int* 2014;**25**:399–405.
12. McInerney-Leo AM, Marshall MS, Gardiner B, Coucke PJ, Van Laer L, Loeys BL *et al.* Whole exome sequencing is an efficient, sensitive and specific method of mutation detection in osteogenesis imperfecta and Marfan syndrome. *Bonekey Reports* **2**, Article number: 456 (2013); doi:10.1038/bonekey.2013.190.
13. Li G, Yu J, Wang K, Wang B, Wang M, Zhang S *et al.* Exome sequencing identified new mutations in a Marfan syndrome family. *Diagn Pathol* 2014;**9**:25–32.

14. van Dijk FS, Byers PH, Dalgleish R, Malfait F, Maugeri A, Rohrbach M *et al.* EMQN best practice guidelines for the laboratory diagnosis of osteogenesis imperfecta. *Eur J Hum Genet* 2012;**20**:11–19.
15. Forlino A, Cabral WA, Barnes AM, Marini JC. New perspectives on osteogenesis imperfecta. *Nat Rev Endocrinol* 2011;**7**:540–557.
16. Sule G, Campeau PM, Zhang VW, Nagamani SCS, Dawson BC, Grover M *et al.* Next-generation sequencing for disorders of low and high bone mineral density. *Osteoporos Int* 2013;**24**:2253–2259.
17. Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. *Nat Rev Genet* 2013;**14**:295–300.
18. Mornet E. Hypophosphatasia. *Best Pract Res Clin Rheumatol* 2008;**22**:113–127.
19. Mornet E, Hofmann C, Bloch-Zupan A, Girschick H, Le Merrer M. Clinical Utility gene card for: hypophosphatasia. *Eur J Hum Genet* 2014;**22**:e1–e5.
20. Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D *et al.* Enzyme-replacement therapy in life-treatening hypophosphatasia. *N Engl J Med* 2012;**366**:904–913.