Journal of the Endocrine Society, 2021, Vol. 5, No. 4, 1–3
doi:10.1210/jendso/bvaa200
Commentary

Commentary

Gene Testing in Everyday Clinical Use: Lessons from the Bone Clinic

Emma L. Duncan

Department of Twin Research & Genetic Epidemiology, School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London

ORCiD number: 0000-0002-8143-4403 (E. L. Duncan).

Received: 10 November 2020; Editorial Decision: 18 December 2020; First Published Online: 30 December 2020; Corrected and Typeset: 6 March 2021.

Key Words: Idiopathic osteoporosis, familial osteoporosis, bone fragility, genetic analysis, candidate genes, massively parallel sequencing

Clinicians identify disease through a defined process. On meeting a new patient, they take a history and examine the individual, and may order chemical pathology or imaging. If everything aligns the pattern is recognized, the diagnosis made, therapeutic algorithms applied, and the outcome prognosticated, with reasonable confidence. When history, examination, pathology and/or imaging do not quite fit together it takes good clinical judgment to know when to adhere to the aphorism that common things occur commonly (with the corollary that individuals with an uncommon presentation are more likely to have a common disease presenting uncommonly than an uncommon disease) or, conversely, when there is sufficient clinical concern to consider the possibility of a rare disease as the likely underlying pathology.

Within the bone clinic, this dilemma is faced when meeting individuals with profound osteoporosis, extremely low bone mineral density (BMD), and/or low-trauma fractures at an unusually early age. Does this individual have a monogenic cause for fragility, through carriage of a rare, highly penetrant allele of large phenotypic effect, such as a mutation in *COL1A1* and *COL1A2*, or other of the long list of genes associated with bone fragility [1]? Alternatively, is this person's severe osteoporosis polygenic in origin? BMD is a highly heritable quantitative trait and to date more than 500 loci have been associated with BMD at genome-wide significance [2], each with small effect upon the individual's phenotype. Individuals enriched with "low BMD" alleles form the lower tail of the normal curve for BMD distribution within the population. Additionally, these are not mutually exclusive options: the ultimate phenotype even of individuals carrying variants of large effect is still shaped by that individual's polygenic background.

Although discussion about the genetic architecture underlying BMD extremes might be intellectually fascinating, the practical question for clinicians caring for individuals with profound osteoporosis is, when should I consider (and investigate for) osteogenesis imperfecta (OI) or other monogenic cause(s) of bone fragility? Clinical flags include severely low BMD at a young age (noting that low BMD is not invariably a feature of OI [3]), fractures in utero, vertebral fractures in childhood, deafness, valvular disease, and genotype-specific clinical features [1], along with a family history consistent with typical inheritance, although this can be difficult to discern for recessive forms of OI and perplexing in X-linked OI, and individuals with de novo mutations will not manifest such a history.

However, increasingly many individuals attending a specialist clinic will have some sort of genetic testing available for interrogation. Millions of people worldwide have already been genotyped by microarray (e.g., through ancestral tracing); and variants with minor allele frequency

ISSN 2472-1972

down to >1% will have been genotyped or imputed with high accuracy, enabling calculation of polygenic risk scores for a host of disorders [4]. Some microarrays include curated exonic content (i.e., rarer variants of clinical import) extremely useful for diseases caused by 1 or only a few variants (e.g., hemochromatosis). Genotyping will be less useful for those rare diseases in which each affected family carries a unique mutation. Instead, such variants will be captured by sequencing, with massively parallel sequencing the ideal technology for screening multiple genes simultaneously, rapidly, accurately, and cheaply [5].

The current paper by Rocha-Braz et al. [6] interrogates a cohort of 28 unique families by panel sequencing, using a panel designed in 2015 of 128 candidate genes. These include monogenic bone fragility genes and genes in or near (<500 kb) loci associated with BMD from genome-wide association studies (GWAS) (as the authors acknowledge, variants within such genes may not necessarily be driving the observed association; accordingly, the authors term these "genes of unknown significance"). Using published guidelines [7], one individual carried a "pathogenic" variant in *COL1A2*, previously associated with OI and/or bone fragility in several case reports. One individual had a variant in *WNT1* categorized as "likely pathogenic", reported previously in compound heterozygote individuals with OI, noting that *WNT1* mutations can be co-dominant [8]. This individual's mother carried this variant and had unusually low BMD also. The authors also report an individual with a "likely pathogenic" mutation in *IDUA*, without evidence of copy number variation. Biallelic mutations in *IDUA* cause mucopolysaccharidosis type 1, with phenotypes including dysostosis multiplex; however, a bone phenotype from single heterozygous carriage is not clearly established [1]. Although in the "likely pathogenic" category [7], this variant was predicted tolerated by SIFT and had low genomic evolutionary rate profiling and combined annotation-dependent depletion scores.

The authors considered whether rare variants in other genes on their panel might contribute to the phenotype of their cases (individually or cumulatively, including in those individuals with pathogenic/likely pathogenic variants). For example, the individual with a *WNT1* variant also had a *PLS3* variant of unknown significance, as did this person's mother. Without functional data, these results can only be considered hypothesis-generating. Additionally, the authors have not genotyped their cohort; whether their cases are enriched with known "low BMD" alleles identified through GWAS, constituting polygenic risk of low BMD, is unknown.

Although panel sequencing has advantages of cheap cost, excellent depth of coverage, and few incidental findings, this study highlights an obvious limitation. The field of monogenic gene discovery has progressed incredibly rapidly: between 2015 and 2019, the number of genes in the Osteogenesis and Decreased BMD Group category of the *Nosology of Genetic Skeletal Disorders* doubled [1], with another 2 genes reported in the 6 months after the most recent publication. Similarly, GWAS have now identified many more loci at genome-wide significance than informed the gene choice here. Thus, with their 2015 panel, the authors could only assess a fraction of the loci of interest they defined a priori. Whole exome sequencing would have allowed the authors to interrogate the genes they were interested in comprehensively, as would whole genome sequencing. This paper also highlights the difficulties in definitive attribution of causality for rare variants, whatever the method of identification. Ongoing and cooperative reporting of phenotype/genotype correlations by clinicians worldwide, along with functional studies, will aid interpretation of sequencing findings.

There seems little doubt that genetic techniques will quite rapidly translate into the clinic. There are potential negative consequences, including genetic discrimination, but this has been addressed by many legislatures. However, the considerable potential benefits include accurate diagnosis, targeting of effective treatment (and avoidance of ineffective options), and genetic counseling. The current paper, which identifies individuals with compelling evidence of monogenic skeletal fragility, illustrates that genetic technologies will improve disease identification and thus are highly likely to become a routine part of clinical care.

Additional Information

Correspondence: Emma L. Duncan, Department of Twin Research, King's College London, St Thomas' Hospital Campus, 3rd Floor South Wing Block D, Westminster Bridge Road, London SE1 7EH UK. E-mail: emma.duncan@kcl.ac.uk.

References

1. Mortier GR, Cohn DH, Cormier-Daire V, et al. Nosology and classification of genetic skeletal disorders: 2019 revision. *Am J Med Genet A*. 2019;**179**(12):2393-2419.
2. Morris JA, Kemp JP, Youlten SE, et al.; 23andMe Research Team. An atlas of genetic influences on osteoporosis in humans and mice. *Nat Genet*. 2019;**51**(2):258-266.
3. Cundy T, Dray M, Delahunt J, et al. Mutations that alter the carboxy-terminal-propeptide cleavage site of the chains of type I procollagen are associated with a unique osteogenesis imperfecta phenotype. *J Bone Miner Res*. 2018;**33**(7):1260-1271.
4. Lambert SA, Abraham G, Inouye M. Towards clinical utility of polygenic risk scores. *Hum Mol Genet*. 2019;**28**(R2): R133-R142.

5. McInerney-Leo AM, Marshall MS, Gardiner B, et al. Whole exome sequencing is an efficient, sensitive and specific method of mutation detection in osteogenesis imperfecta and Marfan syndrome. *Bonekey Rep*. 2013;**2**:456.
6. Rocha-Braz MGM, França MM, Fernandes AM, et al. Comprehensive genetic analysis of 128 candidate genes in a cohort with idiopathic, severe, or familial osteoporosis. *J Endocr Soc*. 2020;**4**(12):bvaa148.
7. Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;**17**(5):405-424.
8. Keupp K, Beleggia F, Kayserili H, et al. Mutations in WNT1 cause different forms of bone fragility. *Am J Hum Genet*. 2013;**92**(4):565-574.