COMMENTARY

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Scrutinizing the Genetic Underpinnings of Bone Strength

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Every year, osteoporosis causes millions of fractures worldwide, with the lifetime risk of suffering a wrist, hip, or vertebral fracture estimated to be about 30% in developed countries.[1] Osteoporosis has been operationally defined for diagnostic and treatment purposes on the basis of the bone mineral density (BMD) assessment performed at the skeletal sites where fracture is most common. Nevertheless, BMD measurement alone is not optimal for the detection of individuals at high risk of fracture. Despite high specificity (ie, risk of fracture is high when BMD is low), BMD measurement also holds low sensitivity (ie, risk is still substantial when BMD levels do not indicate the presence of osteoporosis). This has to do with the multifactorial etiology of osteoporosis and its associated fractures, involving significant environmental influences together with a very large set of genetic factors acting across numerous biological processes. Multiple underlying factors, apart from bone strength, influence the risk of fracture, including age, sex, menopausal status, diet, physical activity, smoking, falls risk, coexisting diseases, and medications. Among the established risk factors, the predictive ability of family history of fracture has led researchers to start looking for the molecular genetic determinants of fracture. Such enterprises usually start by determining how much of the phenotypic variance is explained by genetic factors, ie, determining the "heritability" of the traits of interest. Theoretically, identifying the factors that together constitute the genetic contribution to fracture risk will expand the understanding of the underlying biologic mechanisms, lead to development of novel interventions (treatments), and will enable the application of molecular definitions to reclassify disease and improve risk prediction. Until 2005, the identification of genetic factors for complex diseases such as osteoporosis was plagued with underpowered and irreproducible studies of suspected (well-known) candidate genes in human studies (eg, cases/controls, families, sib pairs, and populations). It was, however, realized that collaboration could overcome several of these hurdles, and this concept proved successful when hypothesis-free interrogations of the complete genome were made possible by novel massively parallel genotyping techniques that analyzed millions of DNA polymorphisms simultaneously. As in other human complex diseases, the field of genetics of osteoporosis has been revolutionized by the advent of the so-called genome-wide association study (GWAS) approach,[2] very rapidly bringing the number of identified BMD loci from none[3] to dozens[4] and currently hundreds[5] in less than a decade of GWAS research. This has resulted in an unprecedented leap in the number of factors and pathways being linked to skeletal biology, some of which have been shown to constitute in retrospect solid leads for pharmacological treatment,[6,7] whereas others revealed clear translational potential of a GWAS discovery.[8,9] The recipe of such success in osteoporosis has been the result of combining BMD (a highly heritable, quantitative, precise, and widely available trait capable of capturing aspects of bone biology) with an ever-growing increase in the sample size of the studies, growing from tenths to now hundreds of thousands of participants (allowing lowering the noise and increasing precision in the process of causative genetic variant identification). Most findings arise from BMD GWAS, predominantly because of the high heritability and widespread data availability of BMD measurements. Efforts focusing on fracture outcomes have been less prolific despite similar sample sizes, which is largely the consequence of lower heritability due to greater environmental influence. Interestingly, to date all the genetic determinants of fracture that have been identified by GWAS are also associated with BMD. Because BMD does not completely capture fracture risk (see above), this observation suggests that approaches other than BMD might yield more genetic factors for fracture. So, does the field indeed need to change the approach and instead of focusing on BMD move toward targeting other skeletal outcomes that can capture better the genetic factors underlying fracture propensity?

In this issue of *JBMR*, Karasik and colleagues[10] have performed a comprehensive determination of the heritability and genetic correlation of bone strength properties in humans, assessed from failure load values determined with micro-finite element analysis (μFEA) measured by high-resolution peripheral

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quantitative computed tomography (HR-pQCT). This work was carried out as part of the Framingham Heart Study[11] in one of the largest samples with assessment of bone microarchitecture. The Framingham Osteoporosis Offspring Cohort, from which the participants are derived, constitutes a two-generational family-based study where heritability can be assessed by determining the variation in a trait, which is explained by the genetic relatedness between individuals in the population.[12] Although heritability estimates do not provide information about the actual variants influencing the variation in a trait, they do provide insight about the underlying genetic architecture of a trait. Even better, when two or more traits are assessed in the same population, the "shared heritability" or genetic correlation can provide further understanding about common biologic mechanisms between traits. This is crucial information for launching genetic investigations into these skeletal traits but also helps in the understanding of the properties of the measurements and biological mechanisms underlying the phenotype they measure, which in turn, can help understand their relationship with fracture.

It has been established that areal BMD (though imperfect in its ability to predict fracture) is a good proxy of bone strength, accounting for up to 80% of the variation in bone strength.[13] As proposed, the remaining unaccounted fraction of bone strength is determined by "bone quality," a composite term referring to structural and material properties, defined as comprising bone mineralization, architecture, turnover, and micro-damage accumulation.[14]

Peripheral quantitative computerized tomography (pQCT) in its high-resolution (HR) and standard versions is a technology able to assess properties that are not generated from areal BMD measurements. With pQCT, volumetric BMD is measured (vBMD), reflecting the actual 3D configuration of bone, and cortical and trabecular bone compartments can be separately assessed. Previous work of the authors in the same study population using HR-pQCT determined that vBMD and bone microarchitecture indices measured at the distal radius (unloaded) and tibia (loaded) bones were heritable and significantly genetically correlated to aBMD.[15] Those findings imply that aBMD remains the trait of choice for the discovery phase of genetic studies of osteoporosis, considering its higher heritability, widespread availability of the measurement (warranting large sample sizes crucial for GWAS), confirmed successful yield of discoveries, and (last but not least) its ability to capture many aspects of bone composition.

In their current work, the authors have now focused on an index of compressive bone strength derived from the HR-pQCT measurements at the ultradistal radius and tibia, which is represented as failure load obtained from micro-finite element analysis. The heritability estimates (h^2 of 42% to 54%) that were obtained for the HR-pQCT-derived failure loads were similar to those of aBMD measured at the same (forearm) or proxy (femoral neck) skeletal sites, thereby indicating that these HR-pQCT traits, like aBMD, may also constitute promising traits for future genetic investigations. Using actual bone strength indices as the outcomes of genetic studies will (at least in theory) provide better understanding of the associations between identified genetic variants and skeletal outcomes. Yet, the failure loads at both skeletal sites were highly correlated with several of the HR-pQCT bone microarchitecture indices and with the aBMD measurements. As expected, the shared heritability (genetic correlation) was also high, reaching 95% between failure load at the radius and forearm BMD and 70% between failure load at the tibia and femoral neck BMD. This means that the variation in genes associated with aBMD are likely to reflect genetic pathways that affect bone strength. Although expected, this observation offers validation of the importance of those identified BMD-associated loci to skeletal pathways and postulates that their effect on fracture, the clinical deleterious consequence of low BMD, is likely because these variants exert an effect on bone strength.

From another perspective, the authors are optimistic that the study of failure load indices has the potential to identify genetic risk factors of fracture, which are independent of aBMD. They base their hopes on the fact that despite not observing significant differences, a trend of change was observed on the heritability estimates after correction for aBMD. Typically, when studying the genetics of complex diseases (including heritability studies), sample size limitations do not allow conclusions to be drawn from only one report. The confidence intervals of the estimates are wide, making it difficult to determine the actual effect of covariate adjustment on the heritability estimates. Then again, heritability studies are liable to misconceptions, many of which also affect the way we interpret their estimates. A given heritability estimate of 50% means that in the sampled population on average 50% of the total variance in failure load can be explained by genetic differences between individuals in a given environment. Therefore, the ethnic background and sex and age composition of participants of the Framingham Osteoporosis Study included in the analysis need to be considered in the perspective of the reported heritability estimates, together with the relevant environmental influences in that population that might differ from those in similar cohort studies conducted elsewhere.

The authors correct their estimates for sex and age to allow generalization, acknowledging their limitation of not being able to stratify and obtain estimates for those specific groups given the resulting power issues. Heritability estimates of complex traits like failure load are not expected to differ significantly between sexes; in contrast, the differences in age and environmental exposures are very relevant and require further study. The mean age of the study population was 72 years, comprising lifelong environmental exposures that are expected to result in underestimation of the lifelong heritability of a trait and indirectly affect the ability to discriminate genetic effects specific to failure load bone strength. Other factors influencing the heritability estimates are related to secular trends being different between populations (ie, physical activity, nutrition, and other environmental factors). Correcting for these factors is not trivial and actually not recommended in heritability studies, as they may result in irreproducible estimates that cannot be easily extrapolated between populations. The sex and age correction applied by the authors suffices for these undertakings, while they also include additional models corrected for body height to establish independence from skeletal size components. They propose that the estimated failure load heritability seems to be independent of bone size as inclusion of height in the models increased the estimates slightly. In fact, inclusion of additional covariates in the models can have the effect of increasing heritability estimates by reducing the overall phenotypic variance of the trait (while also potentially adjusting out variance components arising from genetic shared factors). This reduction of overall phenotypic variance (and resulting slight increase in the heritability estimate) is likely achieved by correction of measurement error captured by the body height adjustment. A note of caution is also needed here for future

genetic studies, as inclusion of heritable covariates in the models can actually result in unintended bias introduced with respect to the primary outcome as a result of the adjustment, ensuing "collider" bias that might lead to false positives (spurious associations).[16,17]

Typically, heritability studies of BMD have shown that measurements of the axial skeleton tend to have higher heritability estimates than those of the appendicular skeletal sites. This is expected to occur in response to differential exposure to environmental factors and also observed among the appendicular sites between the "loaded" and "unloaded" bones.[18,19] Interestingly, in contrast to BMD, the heritability estimates of failure load did not differ significantly between the two sites despite the differences in skeletal loading. This can be interpreted as failure load being a property of bone that is reflecting more the genetic predisposition of bone strength than the environmental influences affecting the skeleton. If this is indeed the case, genetic studies of failure load could also result in a high yield of discoveries when used in GWAS. On the other hand, a very different genetic architecture from that of BMD (comprising less frequent and common genetic variants within genes affecting monogenic and complex forms of the traits)[7] is not expected. Therefore, before the indices of compressive bone strength used in this study can be readily incorporated as outcomes of GWAS, the sample size of studies with HR-pQCT measurements needs to increase several-fold to reach the tens to hundreds of thousands of participants achieved by current successful BMD GWAS.

From this perspective, we can expect that applying the GWAS approach in an ever-expanding number of individuals with the skeletal trait of interest (likely BMD) will continue to allow the identification of hundreds to thousands of genetic variants underlying the risk of osteoporosis and fracture. This approach needs to be complemented by studies performing functional follow-up of the identified loci and their pathways but also by enriching the set of investigated phenotypes that together can help elucidate the processes underlying fracture susceptibility.

Unique opportunities arise in other settings, where the aBMD measurement falls short in advancing the field of genetics of osteoporosis. Thus, the expected gains in knowledge from association studies using aBMD (a single value measurement resulting from a composite of mineral, mass, and size properties of bone) are likely to be limited to detecting the involvement of a gene or genomic region (locus) in bone biology; whereas, combining the knowledge derived from association studies on aBMD with those of the failure load indices (and even the other bone microarchitecture indices measured by HR-pQCT) will increase the understanding of how genetic variation influences specific bone structure and compartments. This will hopefully open translational opportunities in the context of fracture risk etiology. On the other hand, when studying the genetic predisposition of conditions where BMD does not characterize well the actual risk of individuals (eg, fracture risk in diabetes, atypical fractures), studying failure load bone strength can provide valuable insight about the involvement of genes and pathways in the pathogenesis of fracture in those conditions.

Current GWAS discoveries based on BMD are starting to advance the field of genetics of osteoporosis by means of pinpointing drug targets that will potentially lead to the development of improved therapies and preventive measures. A single study of bone strength outcomes is unlikely to yield new discoveries not related to BMD but will definitively provide the basis for extending the understanding of several of these genetic associations with BMD (bone size and density). As previously postulated in this journal, studying and understanding bone strength is not enough.[20] Fracture is not always a result of insufficient strength. Therefore, studies limited to BMD and bone strength will often miss other bone properties that are influenced by genetic factors. This means that studying material failure properties (ie, fracture toughness and fatigue strength) and their associated (molecular) mechanistic pathways is needed. As recently pointed out by Hernandez and van der Meulen,[20] one of the factors limiting the advance of the field is the contention (misconception) that (as described above) the skeletal properties underlying fracture susceptibility can be separated into clearly defined components that act independently from each other. This is one of the reasons limiting the success of genetic approximations, where the mechanistic pathway leading to fracture can be conceived as a "melting funnel pot" of multiple processes that ultimately influence fracture risk, all of which have different degrees of genetic contribution. With fracture risk constituting such a heterogeneous outcome, large samples need to be brought together to identify the real yet weak effects of genetic variants influencing complex traits. Either way, the assessment of those properties not captured by studying bone strength alone needs to be targeted by implementing new technologies and making them widely available, neither of which is easy to achieve in the near future. However, new promising technologies are showing some promise in that direction (ie, MRI identification of bone marrow lesions and bone material strength measured by microindentation). The study of Karasik and colleagues, in determining the heritability and genetic correlation between these novel traits, BMD, and fracture risk, provides a solid start to assess their potential as outcomes for genetic investigations and to understand their interdependencies. Ultimately, the hope is that this approach will be successful in bringing the field further toward fulfilling the expectations of translating genetic discoveries into practical clinical applications.

Disclosures

All authors state that they have no conflicts of interest.

References

1. Cooper C. The crippling consequences of fractures and their impact on quality of life. Am J Med. 1997;103(2A):12S–17S; discussion 17S–19S.
2. Hindorff LA, Sethupathy P, Junkins HA, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A. 2009;106(23):9362–7.
3. Kiel DP, Demissie S, Dupuis J, Lunetta KL, Murabito JM, Karasik D. Genome-wide association with bone mass and geometry in the Framingham Heart Study. BMC Med Genet. 2007;8 Suppl 1:S14.
4. Estrada K, Styrkarsdottir U, Evangelou E, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet. 2012;44(5):491–501.
5. Kemp JP, Morris JA, Medina-Gomez C, et al. Identification of 153 new loci associated with heel bone mineral density and functional involvement of GPC6 in osteoporosis. Nat Genet. 2017;49(10):1468–75.
6. Nelson MR, Tipney H, Painter JL, et al. The support of human genetic evidence for approved drug indications. Nat Genet. 2015;47(8):856–60.
7. Rivadeneira F, Makitie O. Osteoporosis and bone mass disorders: from gene pathways to treatments. Trends Endocrinol Metab. 2016;27(5):262–81.

8. Moverare-Skrtic S, Henning P, Liu X, et al. Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures. Nat Med. 2014;20(11):1279–88.
9. Moverare-Skrtic S, Wu J, Henning P, et al. The bone-sparing effects of estrogen and WNT16 are independent of each other. Proc Natl Acad Sci U S A. 2015;112(48):14972–7.
10. Karasik D, Demissie S, Lu D, et al. Bone strength estimated by micro-finite element analysis (μFEA) is heritable and shares genetic predisposition with areal BMD: the Framingham Study. J Bone Miner Res. Epub 2017 Jul 19. DOI: 10.1002/jbmr.3200.
11. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol. 1979;110(3):281–90.
12. Karasik D, Myers RH, Cupples LA, et al. Genome screen for quantitative trait loci contributing to normal variation in bone mineral density: the Framingham Study. J Bone Miner Res. 2002;17(9):1718–27.
13. Faulkner KG. Bone matters: are density increases necessary to reduce fracture risk? J Bone Miner Res. 2000;15(2):183–7.
14. Sievanen H, Kannus P, Jarvinen TL. Bone quality: an empty term. PLoS Med. 2007;4(3):e27.
15. Karasik D, Demissie S, Zhou Y, et al. Heritability and genetic correlations for bone microarchitecture: the Framingham Study families. J Bone Miner Res. 2017;32(1):106–14.
16. Aschard H, Vilhjalmsson BJ, Joshi AD, Price AL, Kraft P. Adjusting for heritable covariates can bias effect estimates in genome-wide association studies. Am J Hum Genet. 2015;96(2):329–39.
17. Day FR, Loh PR, Scott RA, Ong KK, Perry JR. A robust example of collider bias in a genetic association study. Am J Hum Genet. 2016;98(2):392–3.
18. Kemp JP, Medina-Gomez C, Estrada K, et al. Phenotypic dissection of bone mineral density reveals skeletal site specificity and facilitates the identification of novel loci in the genetic regulation of bone mass attainment. PLoS Genet. 2014;10(6):e1004423.
19. Karasik D, Hsu YH, Zhou Y, Cupples LA, Kiel DP, Demissie S. Genome-wide pleiotropy of osteoporosis-related phenotypes: the Framingham Study. J Bone Miner Res. 2010;25(7):1555–63.
20. Hernandez CJ, van der Meulen MC. Understanding bone strength is not enough. J Bone Miner Res. 2017;32(6):1157–62.