NEWS

SNPs in the Clinic for Fracture Risk Prediction: A Foreseeable Reality or a Distant Dream?

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The genome-wide association (GWA) study has become a familiar player in the bone field. Since publication of the first such study in 2007 (1), additional large studies appearing in prestigious journals (2-4) have provided for osteoporosis researchers results – associations between genetic variations known as single nucleotide polymorphisms (SNPs) and disease – that have intrigued investigators researching the genetic basis of other common diseases like diabetes and Crohn’s disease. In the osteoporosis research arena, investigators have used GWA studies to begin to document reliable associations of SNPs with bone mineral density (BMD), and with fractures.

For many top osteoporosis genetics experts, hypothesis-free GWA studies are most exciting for their ability to identify new genes not previously expected to be involved with osteoporosis. Indeed, in contrast to the candidate gene approach, which seeks associations between genes already known to play important biological roles and disease, GWA studies cast aside previous notions about which particular genes are important in favor of conducting a broad sweep of the genetic landscape. The hope is that the discovery of variations in genes not yet on the osteoporosis field’s radar will lead to a better understanding of disease mechanisms and pathways influenced by those genes.

“The real reason for doing these GWA studies is to identify novel DNA regions that are associated with BMD and fractures,” says Brent Richards, lead author of a GWA study on osteoporosis published in the Lancet (3) last year. “This allows us to improve our understanding of the physiologic mechanisms that impact bone metabolism using a non-hypothesis-based approach that enables us to find things we couldn’t have imagined prior to the study. That, in my mind, is far and away the most important contribution these studies can possibly make,” according to Dr. Richards, expressing a sentiment voiced repeatedly by many of the osteoporosis GWA experts interviewed for this article.

However, in addition to novel discoveries about previously unsuspected DNA regions and the light such new findings cast on disease mechanisms and pathways, one potential application of GWA studies is to use the genetic variants identified to improve fracture risk prediction. However, in contrast to their excitement over the former, there is for most experts great uncertainty over the latter. "I just don't know whether this is going to work or not for fracture risk prediction, and I don’t think anybody really does," according to Matthew Brown, a professor of immunogenetics at the University of Queensland in Australia and a GWA study expert. While some experts – a sizable minority in fact – are indeed optimistic that results from GWA studies will prove useful for predictive purposes and point to several potential advantages of SNPs over other risk factors currently used to predict fracture risk (in fracture risk assessment tools such as FRAX®), the majority of the experts who spoke to BoneKEy remain highly skeptical of GWA studies and instead emphasize the hurdles these studies must overcome.

The Problem of Small Effect Sizes

The SNPs that GWA studies have been examining for associations with disease are common genetic variations. With some notable exceptions, most of these SNPs, including those identified by osteoporosis researchers, have small effect sizes: they alter the risk for a particular disease or trait...
of interest by only a small amount, usually by less than 10%. From an evolutionary perspective, this makes perfect sense. "If something had a profoundly deleterious effect on any trait that had relevance to our ability to reproduce or to survive until the age of reproduction, then it would be selected against, and would not be allowed to become common," notes Dr. Richards, an assistant professor at McGill University in Montreal.

However, what makes sense from the vantage point of evolution makes life difficult for the improvement of fracture risk prediction. "The picture that is emerging from these studies is that the effect of any particular genetic polymorphism on disease risk is generally very weak such that it seems very unlikely that these individual genetic variants will be clinically useful for predicting an individual's future risk of disease," according to Joseph Zmuda, a genetics expert at the University of Pittsburgh who co-wrote an editorial on the Lancet paper when it appeared in May of last year. This view that the associations between SNPs and disease risk are too small to be consequential from a clinical perspective is easily the most common reason why osteoporosis experts who doubt the ultimate contribution GWA studies will make to improve fracture risk prediction are so skeptical.

Since there is strong doubt that any one SNP will impart a large enough increase in risk to meaningfully improve fracture risk prediction, a natural question to ask is whether combining several SNPs, each with a small effect, could actually do so. Tuan Nguyen, a senior research fellow at the Garvan Institute of Medical Research in Sydney, Australia, has tried to address just this question using prognostic models he has developed with his colleagues. His calculations suggest that, even when using multiple SNPs, the ability to discriminate those who will fracture from those who will not is barely affected – he observed only an approximately 1% improvement. "Even if you combine SNPs, they improve prediction only very modestly compared to what we already know about the clinical risk factors," Dr. Nguyen says.

**Reason for Optimism**

Dr. Nguyen, however, is still hopeful about using SNPs to improve prediction, particularly in high-risk individuals, such as those with a strong family history of fracture. Of course, a family history of fracture, which is indicative of the workings of genetics, is already a well-recognized risk factor for fracture, and is included in fracture algorithms such as FRAX®, and some experts say that SNPs would have to add something above and beyond what family history already contributes to risk prediction. What could it add – what advantages, in comparison to this already accepted risk factor, could SNPs bring into the clinic?

"'Family history' is a rather generic term," explains Dr. Nguyen. "It doesn't identify exactly who in the pedigree would have a higher risk. So, if we use SNPs within the family, it would allow us to discriminate between those at high risk and those at low risk within that family," he says. Indeed, Dr. Nguyen has already performed calculations showing that this approach will work for those with a family history of fracture. While he is much less optimistic about the feasibility of using SNPs to screen the general population, he is hopeful that multiple SNPs, used in those with a family history of fracture or in other high-risk groups, and used in combination with other risk factors, is a tactic that will succeed.

Other experts also agree with Dr. Nguyen that family history is a rather blunt tool to assess risk. "Genetic determinants are more precise, can be measured at any time without the need of a living family member, and are also independent of co-morbidities such as old age," according to Serge Ferrari, a bone genetics expert at Geneva University Hospital in Switzerland and BoneKEy editor-in-chief.

In fact, despite the skeptics who point to the small effect sizes of the individual SNPs that have been identified thus far in osteoporosis GWA studies, some experts note that many of the other recognized risk factors for osteoporosis, such as steroid or alcohol use, have similarly sized effects, and thus they also see a future where multiple SNPs, used
in combination with other risk factors, could be used for risk prediction. “SNPs are at least as good as the risk factors we currently use in clinical practice, and I think that’s the main rationale for using them,” according to Tim Spector, senior author of the *Lancet* GWA study and the director of the Twin Research and Genetic Epidemiology Unit at King's College London. Dr. Spector even notes an advantage to the commonness of SNPs: people may be more likely to have them than to have some of the other risk factors. For instance, he points out that the prevalence of the two risk alleles identified in the *Lancet* study is ten times that of corticosteroid use. Dr. Spector also notes that genotyping SNPs will be less expensive than doing BMD tests, or blood tests that measure biochemical markers of bone turnover.

In comparison to other risk factors currently used by the osteoporosis field, SNPs have an additional advantage, according to John Eisman, director of the bone and mineral research program at the Garvan Institute of Medical Research in Sydney and a co-author on recent GWA studies. “The effect sizes observed with SNPs are very comparable to those seen with many other risk factors like exercise, calcium intake and smoking. The difference is that the SNPs, and the risk they impart, are apparent from the beginning, at birth. Therefore the potential advantage is that you can use these genetic factors to plan an approach to preventive health,” says Dr. Eisman, who notes that instead of waiting until damage to bone has already occurred from behaviors whose effects become apparent only later in life, SNPs could alert those at increased risk at a much earlier time.

Part of the resistance to the idea of using SNPs for risk prediction comes from deep, pre-existing ideas about what counts as “genetics,” according to Dr. Eisman. Indeed, he notes that genetics is customarily thought of in terms of mutations that produce extreme phenotypes, such as the collagen gene mutations that result in osteogenesis imperfecta. Anything more subtle – such as a SNP with a small effect size – doesn’t seem as important and able to qualify as a true “genetic” effect. Consequently, acceptance of the use of SNPs for fracture risk prediction may require a shift in thinking away from the traditional view that says extreme effects are the only ones that matter. It may also require an honest recognition of fears related to genetics, particularly the feeling that there is nothing one can do to change one’s genetic inheritance. “There is a general reluctance to accept the genetic determinance of disease – we like what we can modify,” emphasizes Dr. Ferrari.

### Does the Field Need to Move Beyond SNPs?

Attitude adjustments alone, though helpful, will not be enough to bring SNPs into widespread clinical use. For most common diseases, the SNPs that have been identified from GWA studies as being linked to particular traits explain only a small amount of the phenotypic variation observed in those traits. This also applies to GWA studies in the osteoporosis field, as the SNPs pinpointed to date in such studies account for only a small amount of the variation seen in BMD. Experts agree that more of the variation in that trait must be explained before SNPs become useful for fracture risk prediction.

Finding additional SNPs, however, may not be sufficient to bring the fruits of GWA studies into the clinic, because they may not explain enough of the still unexplained variation in characteristics like BMD. However, there are in fact other kinds of genetic variation that may prove able to do so, but the osteoporosis field is only just beginning to explore those other sources. Copy number variation, where there are differences between individuals in the number of copies of particular DNA segments, is one such example, but experts say it is unclear how much variation it will ultimately explain.

“People have high hopes that copy number variation will explain another chunk of the so-called ‘dark matter’ – the remaining genetic variation – but I am not so sure,” says André Uitterlinden, a GWA study expert at Erasmus University Medical Center in Rotterdam in the Netherlands and leader.
of the GEFOS international consortium for the study of genetic variation and osteoporosis. "I think it will contribute to some extent, but it will not explain the complete picture, and it may be in the same category as SNPs in terms of explained variance." Dr. Uitterlinden, who does not envision a significant role for SNPs in the clinic in the near term because they explain too little of the variation in traits like BMD, bases his view on the fact that, while copy number variations occupy a larger area in the genome as determined by the number of base pairs, the number of copy number variations in the genome is only a fraction of the number of SNPs.

DNA methylation, a process where methyl groups are chemically attached to DNA to turn genes on or off, is another potential source of variation. Dr. Uitterlinden is optimistic that these DNA modifications will be found once the technology is in place to do so on a genome-wide scale, but suspects that, like copy number variation, it will explain just a part of the missing heritability in traits of interest.

A final kind of variation that may play an important role is the rare variant. "For fracture risk prediction, I actually think the way forward will not be common variants, because the effects are really small, but rather rare variants. If something is not too rare, but frequent in the population on the order of around 1% but imparts a large effect, that would be helpful in risk stratification," according to Dr. Richards. Also enthusiastic about rare variants is Dr. Uitterlinden, who notes that while current approaches cannot detect variation that is present in less than 1% of the population, this will soon change with the advent of new technology, and he thinks that the osteoporosis field will witness excellent progress in this area over the next 2-3 years. Ultimately, though, experts say it is simply too early to tell how important rare variants, as well as copy number variation and DNA methylation, will be in explaining heritability, and therefore it also remains unknown whether these different kinds of variation will help to improve fracture risk prediction.

Indicative of the complexity in translating GWA studies into the clinic is that there are even two further areas of study that may help to explain the variation observed in traits that interest disease researchers: interactions between genes, and interactions between genes and the environment. Regarding the latter, experts believe that future studies could provide some truly intriguing results.

"The apparently small contribution of a given genetic variant to a trait, or to the risk of disease, could be magnified or even disappear in a specific environment," notes Dr. Ferrari. "Candidate gene approaches have suggested this phenomenon could indeed play a crucial role in several common diseases. In fact, some experts actually think, quite provocatively, that genetic effects on common diseases do not exist per se, but rather only modify our response to environmental factors. In that sense, SNPs could help by defining individual risk in subjects with low levels of physical activity or smoking, for instance, whereas they would not affect risk in those who exercise or do not smoke." This is another example, Dr. Ferrari notes, of how SNPs may be useful for prediction in particular subgroups of individuals who also have other clinical risk factors.

However, while both gene-environment and gene-gene effects could be very important in explaining heritability of traits, the osteoporosis field has barely scratched the surface of understanding them. If these types of effects are important, they could make risk prediction relying on a new SNP-based genetics even more challenging.

Fractures or (Something) Else?

The potential difficulties and complicating factors do not end there. In fact, the osteoporosis field has even more basic work to do: documenting more specific links between SNPs and fractures, the latter of most relevance since BMD serves only as a surrogate for fractures. The challenge in documenting associations between SNPs and fractures stems not only from the limited numbers of fractures included in GWA studies, but also because of the variety of
fractures and confusion over how to best characterize them.

“There is no large consensus among the bone field about what constitutes the true osteoporotic fracture type,” Dr. Uitterlinden says. “We know a few types – hip, wrist, and vertebral fractures – but what the best representative is, and the way they should be defined in large epidemiological studies, are matters of debate.”

While the field pursues clarity in this regard, it is already apparent that the main phenotype the bone field is currently looking at – fracture – is itself exceedingly complex. In fact, experts note that fracture is best characterized not as a phenotype, but rather as a final clinical outcome of a complex set sub-phenotypes including bone geometry, bone strength and others. Which of these will turn out to be most important is unclear. “I think the breadth of phenotypes that people are looking at indicates the uncertainty in the field as to the best way forward,” says Dr. Brown.

Steven Cummings, director of the San Francisco Coordinating Center at the California Pacific Medical Center Research Institute and an expert on osteoporosis disease risk, agrees that the field is still grappling for a phenotype. “The best ‘phenotype’ is not clear,” he says. “The current strategies necessarily rely on what we can measure in many people – BMD and clinical fractures – not what is necessarily determined by genetic variation. But it’s the best we can do.” Dr. Cummings further suggests that the bone field may even need to look beyond its traditional purview. “Genetic variants for rate of ‘aging’ may be as or more important for risk prediction and risk reduction as bone ‘genes’ since fracture risk is so strongly determined by age,” he says. Other experts say that the search for variants that affect a person’s response to pharmacologic treatment may also one day play a large role in the osteoporosis field.

An Uncertain Future? Join the Club!

In 2007, Nature published a landmark GWA, from the Wellcome Trust Case Control Consortium, which found associations between SNPs and 7 major diseases – osteoporosis not among them. Though a bit late to the fray, the osteoporosis field has now clearly joined the ranks of other areas of medicine for which GWA studies are an accepted feature of the research landscape. But now that it is a full participant in this new genetic endeavor, is it special, particularly in regard to using SNPs to improve risk prediction? Experts say no: they emphasize that many of the challenges facing the field – small effect sizes, missing heritability, a lack of understanding of gene-gene and gene-environment interactions, and the like – are identical to those seen in other common diseases, where researchers are also hoping to use SNPs in the clinic. As it stands now, osteoporosis researchers have joined the GWA club, but is it a club where everyone is wearing the same shirts?

References

