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NEWS

Genome-wide Association Studies in the Osteoporosis Field: Impressive Technological Achievements, but an Uncertain Future in the Clinical Setting

Recent IBMS BoneKEy Webinar Focused on the Future Role of GWAS in Improving Fracture Risk Prediction

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Since the completion of the Human Genome Project in 2003 and the first phase of the International HapMap Project in 2005, the genome-wide association study (GWAS) has become a mainstay of biomedical research, with new studies continuing to appear every week in prominent journals across a variety of fields. Indeed, as of June 2010, according to the catalog of published GWAS provided by the National Institutes of Health's National Human Genome Research Institute, 904 GWAS documenting associations, at the $\leq 5 \times 10^{-8}$ threshold considered the standard for statistical significance in the GWAS community, between genetic variations and disease traits had been published in the scientific literature. Alzheimer's disease, prostate cancer, inflammatory bowel disease, obesity, stroke, diabetes, gallstones, cholesterol levels, psoriasis – these are just a sampling of the 165 traits that had been the subject of GWAS since the first investigations were published in 2005.

Not to be left out in the genetic cold, the bone field could finally boast of its own GWAS in 2007, when the first such study of osteoporosis was published, and can now point to several GWAS and meta-analyses of GWAS documenting associations between various single nucleotide polymorphisms (SNPs) and bone mineral density (BMD). From the perspective of sheer technological achievement – finding genuine, strong and replicable genetic associations through examination of hundreds of thousands of genetic markers, in ever larger population samples, in just a single study – the osteoporosis GWAS endeavor has been quite a success. Unfortunately, translating these technological advances into meaningful clinical gains, particularly with regard to the improvement of fracture risk prediction, appears as a more daunting challenge, and the route to realizing this promise is likely to require an approach that differs from the path taken in most previous osteoporosis GWAS. Such was the main theme that emerged from The Clinical Potential of Genetic Markers of Osteoporosis, the fourth IBMS BoneKEy Online Forum (available here). This late-September 2010 webinar focused on the ways that one of the most hoped-for rewards of osteoporosis GWAS – tangible improvements in the ability to identify individuals at greater risk of fracture – may finally be realized.

A Clear Victory for Cutting-edge Technology

That osteoporosis would prove fertile ground for a relatively new technology like GWAS makes sense when considering the degree to which the phenotypic variation in this disease can be attributed to genetic factors, a measure known as heritability. Indeed, not only is about 75-80% of the variation in spine BMD explained by genetic factors, but other osteoporosis-related traits, such as the rate at which bone is lost, the amount of muscle mass, and the level of bone's collagen crosslinks in an individual are all, to a large extent, under genetic control. The GWAS approach holds great appeal for understanding genetic variation in such traits because if offers a way to survey a large swath of the genome in an efficient, cost-effective manner using sample sizes that are large enough to detect associations between

SNPs and the traits of interest. “GWAS chips have enabled us, with a single DNA sample, to survey very large numbers of genetic markers in incredibly efficient ways we couldn't really imagine a few years ago, and to give us extremely reliable results with 99.97% repeatability across the genome,” Tim Spector, main presenter of the webinar, told the BoneKEy audience listening to the webinar live. “We used to get very excited if we covered a few hundred markers, but now it is routine to cover between half-a-million and a million markers, and all for less than about 300 dollars,” according to Dr. Spector, a professor of genetic epidemiology at King's College, London who has been studying the genetic determinants of osteoporosis and other diseases as director of TwinsUK, a registry of approximately 12,000 adult twins in the UK started in 1993.

As one illustration of the technological prowess of the GWAS approach, Dr. Spector noted that SNP associations with BMD documented in GWAS come at very precise levels of significance, usually at p-values of 10^{-8} , which he stressed is more than a million times better than the p-value of .05 that is viewed as the minimum level of significance acceptable in clinical studies of drug effects, for instance. “This means we get very robust results that are replicated, which is not the case with a lot of the past genetics that most of us in the field have worked with and published on erroneously,” Dr. Spector said.

Indeed, the superior technology of GWAS has revealed that the “past genetics” – especially candidate gene studies that focused on genes already known to play important roles in bone biology – has often provided spurious results. For instance, Dr. Spector cited a 2009 study he published with colleagues in the *Annals of Internal Medicine* meta-analyzing GWAS that had been conducted in 5 large cohorts to test whether associations documented in previous candidate gene studies, which had faced a number of limitations such as insufficient sample sizes and the absence of replication groups to verify initial findings, were in fact real. With the approximately 36,000 SNPs – all the HapMap SNPs in or near 150 previously published candidate genes – as their purview, the authors came to a disheartening conclusion: the overwhelming majority (94%) of candidate genes did not contain any common variants associated with BMD; rather, just 9 of them harbored variants associated with this trait. “This is a salutary lesson that's also been repeated in other common diseases like diabetes,” Dr. Spector said. “Most of these [previously reported] associations are not real.” Regarding this issue, Serge Ferrari, BoneKEy editor-in-chief and moderator of the webinar, emphasized that the nature of the population samples included in GWAS may explain this outcome. “GWAS gain in power because of large sample sizes, but these samples are more heterogeneous in terms of ethnic background, phenotyping, and environmental variance, so signals found in the more homogenous cohorts of smaller size characteristic of most candidate gene studies could be lost,” according to Dr. Ferrari, who also noted that an association considered as non-significant in a GWAS could be a false negative, that is, it would have been a true association if fewer tests had been performed such as in a candidate gene study.

Still, regardless of this important caveat, Dr. Spector argued that unlike past candidate gene study results, faith in GWAS findings is much stronger because of the ability of this technology to cover much larger numbers of SNPs, and in much larger sample sizes (particularly through use of meta-analytic approaches). In support of this exceptional technological capability, Dr. Spector emphasized that GWAS has already successfully identified genetic variation in genes whose biological importance is so great such that drugs have already been developed to target the pathways those genes control. For instance, he noted that 3 of the top 6 associations pinpointed by GWAS are variations in *RANKL*, *OPG* and *ESR1*; denosumab has already been developed to target the RANKL/OPG pathway, while hormone replacement therapy and selective estrogen receptor modulators have been used to target the estrogen pathway. In addition, other “hits” from GWAS also emanate from genes regulating pathways that already have drugs focused on them, including variations in

SOST, which regulates the sclerostin pathway for which an anti-sclerostin antibody is currently being developed; the farnesyl diphosphate synthase gene (*FDPS*), which encodes an enzyme involved in a pathway that bisphosphonates already target; *PTH*, for which there is also an osteoporosis drug; and *CTSK*, which regulates the cathepsin K pathway for which the cathepsin K inhibitor odanacatib is being developed. Thus, out of the approximately 3-dozen associations from osteoporosis GWAS discovered thus far, "we've got a dozen that we already know are of extremely important biological value, so I think it's very important to note what these scans do deliver, and certainly osteoporosis is one of the best examples we've got," Dr. Spector emphasized.

Of course, the goal of GWAS is not just to confirm the usual suspects but to identify new variants as well, and here too osteoporosis GWAS has had some success. For instance, using meta-analysis of the same 5 cohorts that had been studied to test the 150 candidate genes, an international consortium known as GEFOS (GEnetic Factors for OSteoporosis) identified 8 novel loci for lumbar spine BMD, and 7 novel loci for femoral neck BMD. In addition, Dr. Spector is optimistic that by using larger sample sizes, especially through meta-analysis, and by analyzing phenotypes other than bone density, investigators will likely identify many new loci.

GWAS and Fracture Risk Prediction: A Substantial Challenge for the Bone Field

In contrast to the formidable technological achievements of GWAS in the osteoporosis arena to date, and as is the case for most other common diseases that have been studied by this technique, the translation of GWAS results into the clinic – in the bone field's case, into an improvement in the ability to predict which individuals are likely to fracture – appears as a more daunting endeavor. Why this is so, and how future genetic studies can begin to provide more useful information for clinical purposes, served as the focus for much of the webinar's panel discussion.

The primary reason why the potential translational rewards of GWAS appear distant is that the genetic variants that have been identified to date explain only a minute amount of the phenotypic variation in BMD – just 2%, in fact; with all of this "missing heritability" still to be discovered, the bone genetics field has a lot of work ahead of it. As it stands now, the genetic variants identified by osteoporosis GWAS thus far have small effect sizes: they increase the risk of fracture only by about 10-50%. While Dr. Spector noted that this small increase in risk nonetheless approaches that seen with well-known epidemiological risk factors for fracture like smoking and use of glucocorticoids, the difference is that one can intervene on epidemiological risk factors – the patient can try to give up cigarettes, for instance – while an appropriate intervention on a genetic risk factor is more difficult to identify.

Furthermore, considering these limitations, the enthusiasm for personal genomics amongst the general public has run far ahead of its actual clinical utility. Indeed, companies like 23andMe, which will genotype the DNA of those curious about their genetic risk of disease (and willing to pay $429.00 for the service), are as yet unable to provide data that are actually useful from the perspective of risk prediction, according to panelist Cecile Janssens, an expert in clinical and public health genomics at Erasmus University Medical Center in Rotterdam, The Netherlands. "[Such genetic analysis] is not predictive at the moment, and one's risk keeps changing as long as new variants are being discovered, and as the risk model is updated with new variants an individual's risk might easily change, so I really think it's non-informative at the moment," Dr. Janssens said. In fact, rather than for risk prediction of a trait like fracture, Dr. Janssens is more optimistic about a more limited use of gene markers for predicting which individuals are most likely to respond to which specific treatments. In fact, just one or a few genetic markers with strong effects in the particular therapeutic pathway that a drug targets may be all that are necessary to enable targeting of the right medications to the right people. Unlike these

pharmacogenomic applications, however, using gene markers to improve fracture risk prediction is not in the immediate future.

What Is the Best Path Forward to Improve Fracture Risk Prediction?

Common variants in the general population or rarer variants in subgroups?

Considering the missing heritability, and the small effect sizes of variants identified by GWAS so far, Dr. Ferrari asked the panelists whether the common genetic variation that has been the focus of GWAS efforts to date may be the wrong place to look if improving fracture risk prediction is the goal. “In the osteoporosis field we need to focus much more effort on trying to find lower frequency or rare variants that have a much greater impact on the individual patient's risk of osteoporosis,” according to panelist Joseph Zmuda, an expert in the genetic epidemiology of osteoporosis at the University of Pittsburgh. To illustrate the potential payoff of an approach that focuses on less common variation, Dr. Zmuda pointed to lower frequency and rare variants that have been identified in the LDL cholesterol receptor that make those who have them much more likely to have abnormal cholesterol and a higher risk of developing coronary heart disease. “Several hundred lower frequency or rare variants in the LDL receptor have been identified that don't contribute a lot to the overall phenotypic variation, but to the individual patient they have a huge impact on his or her phenotype and therefore are very relevant clinically,” Dr. Zmuda said. While just a few years ago it was prohibitively expensive to sequence rare variants, costs have come down drastically such that it is now a realistic possibility.

It is also the rare variant that may significantly improve the predictive capability of FRAX®, the WHO fracture risk assessment tool for the bone field. One of the appealing things about using genetic markers for fracture risk prediction is that in many ways they would have advantages over how genetic factors are incorporated into FRAX®. Currently, FRAX® incorporates genetics through its inclusion of family history, one of the strongest risk factors for fracture, as one of the risk factors it uses to calculate an individual's ten-year probability of fracture. However, Dr. Ferrari emphasized that family history is a less-than-ideal measure; for instance, it requires that the patient have a parent who lived long enough to have a fracture, whereas genetic markers could be used without this requirement, and at a much earlier time point in the patient's life. Dr. Spector noted that it is not cost-effective at present to use the common genetic markers identified so far by GWAS to improve the FRAX® calculation. However, he stressed that in the future it is really the use of rare variants, because of their larger effect sizes, rather than common variants, which will offer the greatest potential to improve the predictive capabilities of FRAX®.

Because rare variants, by definition, are less prevalent than common ones, it is reasonable to wonder whether it might prove very difficult to find them. However, Dr. Spector noted that, according to calculations his group have performed, because rare variants will have larger effect sizes than common variants, a couple of hundred cases and controls may be all that are necessary to uncover an effect size as large as 3-fold, relieving the burden of gathering very large sample sizes. This is particularly true if the focus is placed on subgroups of people – particular families, for instance, or people living in specific geographic regions – likely to harbor the rare variants.

Subgroups: this is where the ultimate payoff of GWAS in the osteoporosis field may reside. Indeed, Dr. Ferrari noted that rather than improving risk prediction in the large numbers of people likely to suffer from typical forms of osteoporosis, these genetic investigations may instead be of particular predictive value for subgroups of people with more atypical forms of the disease, such as, perhaps, men suffering from idiopathic osteoporosis, or women dealing with more severe forms of postmenopausal osteoporosis.

Another kind of subgroup – that defined by ethnicity – may also play an important role in future studies of human osteoporosis

genetics. The GWAS of most common diseases, including osteoporosis, have been conducted primarily in Caucasian European populations, but ethnic subpopulations such as Asians and African-Americans may have much to teach us. However, while it is clear that genetics does account for some of the ethnic differences seen in the risk of osteoporosis, it is still unclear what the specific genetic architecture of any particular ethnic group will turn out to be. “It may be possible that there are different genetic variants that may be involved in different populations, but it's also possible that a given variant may have a different frequency in the different ethnic groups. We need more data to know the answer to this question,” according to Dr. Zmuda, who said that the osteoporosis field will likely benefit greatly from studies of lower frequency and rare variants in the various ethnic subgroups.

Gene-environment interactions

While a focus on less common genetic variation, and on subgroups, is likely to be the basis of a large part of future osteoporosis genetic studies, panelists agreed that studies that focus on genetics alone may be insufficient to carry GWAS findings into the clinic; incorporating environmental factors, and in particular interactions between genetic and environmental factors, is the direction in which investigations not just in osteoporosis but in other common diseases is now heading. However, this more holistic approach poses challenges too. For instance, despite the efforts of large consortia, documenting gene-environment interactions with large effect sizes has been difficult, according to Dr. Janssens. “When gene-environment interaction is found, it's of the same magnitude as the [effect of the] gene variant itself,” Dr. Janssens said. In addition, she noted that the gene-environment relationship is likely to be a difficult one to disentangle, with different numbers of genetic factors likely to interact with different numbers of environmental factors in different people. “I think the interaction is far more complex than an interaction between a single gene and a single environment,” Dr. Janssens emphasized.

Interestingly, genetic studies of mice have already shown interactions between genes for BMD and the environment – in the latter case, in the form of dietary patterns – demonstrating the continued relevance of this animal species even in an age of human genetics. In fact, it was a fortuitous circumstance that led to this outcome, according to panelist Cheryl Ackert-Bicknell, a mouse osteoporosis genetics expert at the Jackson Laboratory in Bar Harbor, Maine. Dr. Ackert-Bicknell told the audience that about half of the studies related to genetic variation in BMD in mice occurred in animals fed a high fat, orexigenic diet, as the original purpose of studying such populations was to understand body composition and its relation to serum lipids; discovering regions of the genome (called quantitative trait loci) linked to BMD in these mice was in fact a happy byproduct of this original intent. “This has been a real boon for us in the osteoporosis field, because we can actually start looking at these environment by genetic associations with a great deal of power, and we have actually uncovered quite a few diet-oriented factors that impact upon BMD,” according to Dr. Ackert-Bicknell. Indeed, Dr. Ackert-Bicknell pointed to research showing that the genetic signal picked up in a study can differ depending upon the diet that the animals are fed. In the future, mice may also allow researchers, in some instances, to test specific association signals detected in human GWAS in different environments, again to assess whether gene-environment interaction is at play.

Phenotypes

Another way to increase the chances that GWAS findings will eventually help to improve fracture risk prediction, Dr. Ferrari suggested, is to focus on more relevant, better-defined phenotypes, and the panelists agreed. Thus far, the osteoporosis field has focused on BMD, but panelists agreed that this phenotype has limitations. “I'm beginning to wonder if BMD, whether measured by DXA or CT, is the right phenotype, and whether it's actually a physiologically-regulated phenotype. For example, BMD is a composite phenotype derived from bone mineral content and the

size of the skeleton, and those can be viewed as very different physiologically-regulated traits," Dr. Zmuda said.

Of course, BMD is merely a proxy for the phenotype of most interest to osteoporosis researchers – fracture – but fracture too is far from an ideal phenotype for genetic studies not only because it is often not well-defined, but for other reasons as well. “Fracture is a very difficult phenotype to study – it's not simply a bone-related phenotype,” according to panelist David Karasik, director of the genetic epidemiology program at the Institute for Aging Research, Hebrew SeniorLife in Boston. For instance, one factor unrelated to bone but important to fracturing is falling, which is in fact the prerequisite for most osteoporotic fractures, and risk of falling depends on a number of variables such as muscle strength, balance, and the use of medications such as benzodiazepines. “We might gain better signals from genetic studies by examining more refined phenotypes, with more refined imaging techniques,” Dr. Karasik said, citing bone geometry assessed with techniques like microCT as one possibility.

Finally, because many of the genes for common diseases have pleiotropic effects, where the same gene affects different phenotypes, it will be important for the bone field to consider GWAS of related phenotypes. Such investigations have already been carried out recently for circulating 25-hydroxyvitamin D concentrations, and for age at menarche and age at menopause, and have the potential to reveal new variants that may also be indirectly associated with osteoporosis risk.

A New Perspective

Ultimately, the promise of GWAS to improve fracture risk prediction will likely be realized only with shifts in perspective: from the common variant with a small effect to the rarer one with a larger effect; from the genetic marker considered in isolation to the one considered in relation to environmental factors (and also in relation to other genetic markers); from the phenotype of BMD to a more suitable one like bone geometry and microstructure. Benefiting from these new vantage points, GWAS still has great potential to impress us not just with the strength and sophistication of its technology, but also with its power to provide tangible benefits to patients in the clinic.