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## *PERSPECTIVES*

# Meta-Analysis of Osteoporosis Genetic Studies – Much Ado About Nothing?

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**Abstract**

Meta-analysis is an increasingly used tool in many areas of biomedical research. There are very few fields of research where there is no conflict in the published literature, or where there are unarguably definitive studies available. This is particularly the case in genetics, where, for reasons outlined elsewhere, current approaches have been plagued by high false discovery rates and inadequate power. Not surprisingly, some investigators have turned to meta-analysis to address these problems. This review examines the strengths and weaknesses of the different forms of meta-analysis, with a particular focus on their record and potential future role in osteoporosis genetics. *BoneKEy-Osteovision*. 2006 July;3(7):10-14.


The 'evidence-based medicine' movement has anointed meta-analysis of therapeutic interventions as being of higher standing than individual, large, randomized control trials (1). Is this really justified, and can this valuation be generalized to genetic studies? The record in osteoporosis genetics indicates that we should treat the findings of even large, well-performed meta-analyses with caution, as they are not immune to biases, and have not infrequently produced contradictory results.

The 'big three' genes which have attracted the vast majority of attention to date in osteoporosis genetics encode the vitamin D receptor (*VDR*), type 1 collagen alpha 1 chain (*COL1A1*) and the estrogen receptor alpha (*ESR1*). Each of these has had more than one meta-analysis performed of it, with no great increase in clarity as to their role in determining BMD or fracture risk. A retrospective meta-analysis of *ESR1* variants and BMD and fracture risk by Ionnadis and colleagues reported the findings of studies of 5834 women from 30 study groups (2). The study reported association of XX homozygotes (the minor allele of SNP rs9340799) with increased BMD at both the femoral neck and lumbar spine. There was no evidence of heterogeneity between studies, and publication bias checks, including inverted funnel plots and recursive cumulative meta-analysis, did not indicate the presence of any bias. Funnel plots primarily examine the relationship between study size and reported significance; cumulative meta-analysis examines whether the observed significance changes with increasing data availability (initial studies typically report the highest level of significance). A significant reduction in fracture was also observed amongst XX homozygotes (odds ratio 0.66, p=0.017). The authors concluded that future research should examine the effect of other polymorphisms, including those potentially in linkage with the rs9340799 SNP. Nonetheless, in 2004 Ionnadis and colleagues performed a prospective meta-analysis of rs9340799, rs2234693 (*Pvu*II; also studied in the previous paper), and a dinucleotide repeat in 18,917 men and women from 8 European centers (3). The conclusion from this study was that rs9340799 affected fracture risk, but not

bone density, leading to speculation that the mechanism of association with fracture was through bone size effects rather than BMC.

Both these studies were of high quality, and the differences in findings are not due to methodological errors, but demonstrate that meta-analysis, particularly when performed retrospectively, is prone to error. The current consensus with regard to *ESR1* and its role in adult bone density and fracture risk is that it probably plays a role in fracture risk, but whether this is through effects on bone size or on BMC is unknown. Only a small proportion of the genetic variation in *ESR1* has been studied, and there is still a need for a systematic study of the gene, just as there was prior to either of these meta-analyses.

A similar experience has occurred with meta-analysis of *VDR*. As with most genetic findings, the initial reports of strong association of *VDR* polymorphisms with BMD have subsequently been tempered by failure of replication, or even completely contradictory findings. The reasons for this include genotyping error, error of statistical analysis, incorrect zygosity assignment in twins, and the vagaries of chance, which play a particularly important role when studies are underpowered. Meta-analysis addresses the issue of sample size, but generally not the other sources of error. There have now been at least five meta-analyses of *VDR* variants, with results almost as varied as the studies they have pooled (4-8). The role of *VDR* genetic variation in adult BMD and fracture risk has therefore not been clarified by this approach, other than to indicate that if the gene does play a role in BMD, then the variants that have been studied have a very minor effect at most. Recent studies in very large cohorts have demonstrated no significant association of *VDR* polymorphisms and BMD, bone loss or fracture, confirming what most people in the field believed (9).

The Sp1 polymorphism of *COL1A1* (rs18000120) has been examined by both retrospective (10) and prospective meta-analysis (11), with both suggesting association with BMD at the femoral neck, and at the lumbar spine but with a smaller effect size. The retrospective analysis suggested a highly significant association with fracture (either 'any' or vertebral), whereas the prospective study, although more than three times larger, showed no significant association with fracture. Strangely, neither study reported on hip fracture, the fracture of greatest interest. The prospective study is undoubtedly a 'gold-standard' study, and thus we must assume that the retrospective analysis suffered some form of bias causing the false positive finding with regard to fracture. This again highlights the poor reliability of retrospective meta-analysis.

A key issue in retrospective meta-analysis is how comprehensively the available data has been collected. Data may be found in peer-reviewed publications, abstracts, or even be unpublished. The use of non-peer reviewed data has been much debated, but surveys of authors of meta-analysis papers indicate that the majority support its use. Does publication bias affect osteoporosis genetics, or are investigators driven to publish independent of the significance of their findings? A quick survey of the abstracts submitted to the 2000 and 2001 American Society of Bone and Mineral Research Annual Scientific Meeting shows that only a minority of abstracts make it into the light of day as peer-reviewed publications. In these two years there were 18 abstracts reporting association studies of *VDR* gene polymorphisms and osteoporosis or fracture. Of these, only five have, to date, been published in a peer-reviewed format. As most osteoporosis genetics meta-analyses have not included abstracts, the majority of these studies would not have been included in *VDR* meta-analyses. We have no way of determining how much unpublished data is available, but, particularly in private industry, negative results rarely see the light of day, whereas positive results are widely promoted. The situation with other much studied osteoporosis candidate genes is similar, suggesting that the meta-analyses published to date are far from comprehensive, and thus open to bias.

Meta-analysis has a potential major role to play in linkage studies as well. It could be argued that meta-analysis has an even greater role with linkage studies than with association, given that nearly all significant linkage studies are published, and publication bias is therefore less prevalent. Methods can be divided into either approaches that utilize the actual transmission data within the families studied, and the 'genome-screen meta-analysis' (GSMA) approach (12). In the former method, inheritance-by-descent statistics from different scans are combined either equally (in pooled meta-analysis) or weighted taking into account the differences in size, marker density and linkage information content extracted by each screen. In GSMA, linkage results from different screens are scored in bins of fixed length along chromosomes, and for each study the maximum linkage within each bin is then used to rank the bins within each study from strongest to weakest linkage score. These ranks are then summed across studies for each bin to provide the overall linkage score. The scores can also be weighted, typically in relation to the sample size rather than marker density or characteristics of the families, both of which affect the power of each study but are ignored by this method. GSMA is less powerful than standard linkage meta-analysis (13), but is less complex to perform, and does not require studying the original linkage data, because the ranking in bins is performed using LOD scores or p-values, not the inheritance information within each family. In the author's opinion, GSMA should not be encouraged because it is less powerful, has low resolution (because of the pooling of results into wide 'bins'), and because of its use without obtaining the original data. In such large datasets of cases and markers, errors of marker order, genotyping errors and even changes in affection status amongst cases are not rare. Original linkage data can nearly always be obtained, and allows a further opportunity for error checking, as well as producing significant power gains.

One GSMA linkage meta-analysis has been published in osteoporosis, without the authors requesting the original data (14). Linkage scores were analyzed in 30 cM bins, thus linkages occurring up to 30 cM apart were considered to have come from the same linkage 'signal'. This study found no significant evidence for linkage on a genome-wide scale, despite analyzing data from 3097 families and 12,685 individuals. While this may be a correct finding, it may also relate to the lower power of GSMA approaches, and we await a 'gold-standard' meta-analysis in this field.

With a record like this, can we really hold up meta-analysis as the highest standard of evidence in osteoporosis genetics? Clearly not. The author's personal view is that there is little to be gained by further retrospective meta-analysis of the association data that has been published to date, and that the role of prospective meta-analyses of known candidate genes is limited. Retrospective meta-analysis is too affected by publication bias, and has limited ability to screen out genotyping and other errors from the data set. As the old truism says: garbage in, garbage out. Adequately powered confirmation studies are what are required, but these need substantially larger sample sizes than the discovery studies. Whether these are truly meta-analyses is a semantic issue. Promoters of prospective meta-analysis studies argue that they also provide accurate assessment of the magnitude of the genetic effect. However, where such studies involve many different groups, great care has to be taken to minimize sources of noise, such as effects of varying ethnicity, background environment and sometimes subtle ascertainment effects, causing loss of power and reducing the effect sizes observed.

Genetics has undergone a technological revolution in the last couple of years, with major advances in genotyping technology bringing down costs and improving accuracy and genotyping success rates. Our efforts would be better placed investigating novel genes rather than focusing on the same old genes, where with few exceptions there is

little current evidence to support their having a role over and above the rest of the genome. Both discovery and confirmation studies should have adequate power to detect realistic genetic effect sizes, and discovery studies should attempt to capture a large proportion of the overall genetic variation in the gene being studied. Genomewide association studies are underway in several diseases and are planned in osteoporosis. There is an ethical argument that all data from genetic studies should be made publicly available to bona fide researchers in anonymized format. Full data release maximizes its utilization, and therefore the benefit to the funders, study participants, and affected individuals. Several genetics groups, such as the Wellcome Trust Case-Control Consortium (http://www.wtccc.org.uk/), a publicly funded consortium performing genomewide association studies in a variety of common polygenic diseases, are doing just that. Similar transparency is commonplace in other areas of biomedical research such as clinical trials, microarray gene-expression studies, and in many statistical journals, with reports of new analysis programs. The author feels that this approach should be universal with large osteoporosis genetics studies too, even for targeted genotyping, and that funding agencies and journals have a role to play in ensuring that this happens.

The major weakness of genetic meta-analyses is that they can only look at what has gone before. The problem with respect to osteoporosis genetics is that we have really only touched the tip of the genetic iceberg in the studies done to date. We need to move forward and put our efforts into designing and performing studies which cover the vast majority of the genome that we haven't looked at yet. The last decade has been a steep learning curve in osteoporosis genetics, but the technology, study designs, and understanding of the structure of the human genome and its diversity have improved markedly in that time. Why spend time and resources delving into genes that we already know have only small effects, when we have the tools and opportunity to do so much better?

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