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COMMENTARIES

Towards Genomewide Association Studies in Osteoporosis – Lessons from Early Scans

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Commentary on: 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 Sep 19;8 Suppl 1:S14.

There is little doubt that 2007 is the year that complex human genetics has come of age, with the emphatic demonstration that genomewide association studies are capable of identifying many genes involved in common diseases. Phenomenal advances have been made in the genetics and, as a result, our understanding of the etiopathogenesis of many common diseases, providing basic researchers with solid foundations from which to pursue hypothesis-based research. It is a disappointing fact that such studies of osteoporosis have lagged well behind the progress being made in most other major common diseases, something that can only be ascribed to funding issues given the wealth of well-characterized cohorts available for genetic interrogation of determinants of osteoporosis risk. Doug Kiel and colleagues partially redress this deficiency in their study reported in the September 19th edition of *BMC Genetics* (1).

In this study, 1141 individuals from 241 families were genotyped using an early generation Affymetrix microarray genotyping chip, which typed roughly 113,000 SNPs. The individuals studied had been extensively characterized for bone phenotypes, with analysis for 10 primary variables reported here, and presumably others analyzed but yet to be reported. The data were analyzed for both linkage and association. Association analysis was performed both 'within-family' using the program FBAT, and on a population level using the 'generalized estimating equation' (GEE). The significance of this is that while FBAT is robust to inflation of statistical findings due to population stratification, it is less powerful than the population association approach, which is, however, susceptible to population stratification. Two regions (on chromosomes 15 and 22) were found to be linked with one phenotype (femoral section modulus) with a LOD score of >3.0, but no region achieved 'significant' linkage, emphasizing the low power of this approach. Twelve SNPs achieved $p < 10^{-6}$ by GEE, and 2 by FBAT, but none achieved experiment-wise significance. Several candidate genes for osteoporosis showed modest evidence of association including *MTHFR*, *ESR1*, *COL1A1*, *LRP5*, *VDR*, *PPARG*, and *CYP19*. There was little evidence of pleiotropy (*i.e.*, associations tended to be observed with single phenotypes). There was little correlation between the association findings and the linkage results, with no associated gene clearly explaining any of the observed linkages.

This is an early and valiant effort to perform a genomewide association study, from which the investigators no doubt learned much of value towards the performance of definitive studies in the Framingham cohort, which are currently close to completion. Of course it is disappointing that this preliminary study did not robustly identify any novel genes for osteoporosis, but given the study design, that would have been quite unexpected. Future studies will have a much better chance of making progress.

A considerable strength of the study is that the authors have made all the genotype data available to the genetics research

community. This is an extremely positive trend initiated by the Wellcome Trust Case Control Consortium, which made the genotype data from their landmark studies publicly available earlier this year (2;3). The public availability of the genotype data maximizes its utility, which is of particular importance given the considerable cost of these studies.

There are many limitations of the study that serve as lessons to genomewide association studies. The genomic coverage achieved with this marker set is probably quite low, though not estimated in this study. In fact, only 70,897 SNPs were successfully genotyped. These are randomly distributed throughout the genome rather than being selected to 'tag' regions, and thus have lower coverage than could be achieved through careful marker selection. Assuming all 113K SNPs are successfully genotyped, this chip has been estimated to cover about 30% of the human genome in white Caucasians (4). Given that 37% of SNPs were removed from the analysis for various reasons, the extent of genomic coverage will be significantly lower than that estimate. In our opinion, a genomewide association study should have high coverage (>80% of the genome covered), and at least adequate statistical power to identify genes of the likely effect size present. As the sample size of this study is small, its power is modest, being sufficient to identify only variants contributing ≥4% of the phenotypic variation, assuming no genotyping error. In reality, genotyping error was likely in this study (see comments below), and therefore the power is probably lower than these estimates. Increasing the marker density will not increase the study power, although it will improve its coverage. The message is that unless there are genes of quite large effect sizes operating, the Framingham Cohort lacks adequate power to robustly identify genetic effects. Indeed, for cohorts drawn from the general population, the early experience with many quantitative traits is that >10,000 samples may be required to identify effects robustly. Thankfully, the general willingness of investigators to share data for meta-analysis has made it feasible to achieve those numbers.

The algorithm used in this study to call the genotypes from the Affymetrix chips is known to be particularly inaccurate in calling heterozygote genotypes. Much better algorithms have been developed, and it is surprising that they were not employed in this study (2). One way of selecting out poorly performing SNPs is to remove those that have a high failure rate. In the current study, markers could fail in up to 20% of cases and still be included. This is much less stringent than most current studies and is likely to have led to the inclusion of many poorly performing SNPs. These problems are likely to have contributed to the excess of positive findings observed with the GEE results. For example, the study overall found 19 times as many results as expected by chance at $p < 10^{-7}$. As the Framingham investigators themselves comment, "these findings are best regarded as hypothesis generating," and most will prove to be false positives. Unfortunately, this study has no replication component, and thus even the positive findings are of uncertain significance.

These study weaknesses are discussed frankly and in detail by the investigators (5), also a welcome feature of this study. Several key features from this study should be noted for future genomewide association studies – so that the confusion arising from the past decade of osteoporosis genetics is not carried forward to this new era. These include:

1. Studies should have adequate power to identify the genes of likely effect size operating in osteoporosis and sufficiently dense coverage to properly cover the genome. The study power and estimated coverage should be provided in the study report.

2. There should be a formal assessment of the presence of population stratification. Kiel and colleagues used a within-family analysis to test association unbiased by population stratification, but this had quite low power, and the GEE analysis, which had the better power, is susceptible to stratification. There are now well-developed methods for identifying and controlling for stratification that should be employed.

3. Positive findings should be replicated, preferably as part of the original study. This is particularly important for studies such as Framingham where multiple phenotypes are being investigated in the same individuals. While this is a very efficient design in that one set of genotypes can be tested against

multiple phenotypes, the increased number of tests performed leads to a larger number of false positives. Osteoporosis genetic studies have been particularly guilty in the past of not addressing this issue, and of 'salami slicing' the available data into multiple 'independent' publications. It is to be hoped that we don't have another round of such publications with genomewide association analysis; such behavior simply fuels cynicism about the value of this research and may help explain why osteoporosis has missed the first wave of true genomewide association studies. Kiel and colleagues are to be congratulated for including all the primary phenotype analysis in the one report.

4. Stringent investigation of genotyping error is critical, as these are always over-represented in the most strongly associated SNPs. Where investigators have used liberal genotype inclusion thresholds such as in the current study, there should be verification of genotyping accuracy using another genotyping method. Genotyping error rates should be estimated, by testing genotype known controls and by duplicate genotyping.

5. Genotype data should be made publicly available at the time of initial publication at the latest. We owe this to the public funding agencies who invest in our research, and to the study participants who provide their samples and clinical details to us. The interests of these stakeholders are best served by maximizing the utility of the data through its free availability to bone fide researchers.

The genetics community is well aware of these issues, and we can expect a series of high quality genomewide association studies over the next couple of years, including a proper genomewide association study of the Framingham cohort. It will be interesting to see if these studies produce the same sort of breakthroughs that have already occurred in many other diseases. Even at this early stage, it is apparent that not all diseases are tractable to this type of study, likely because of problems such as genetic heterogeneity and departure from the common-variant, common-disease hypothesis. Genetic heterogeneity is likely to be a problem for osteoporosis studies, but until we test the genomewide association approach in this field it is difficult to know whether phenotypes such as bone mineral density (BMD) are too complex genetically to address by this method. A priori, it seems likely that designs that minimize heterogeneity related to age, gender, site of measurement (of BMD), hormonal status, and potentially environmental background, will be more likely to succeed. As many/most of the cohorts currently being studied are not designed to minimize these issues, it is quite possible that the early genomewide association studies in osteoporosis will not be as productive as we would hope. Cohorts of convenience may be therefore less helpful in unraveling the genetics of osteoporosis than those with recruitment designs aimed to address specific genetic questions. The extent that these issues influence studies of genetics of osteoporosis and other quantitative traits will be played out over the next couple of years.

Conflict of Interest: None reported.

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