

Genetic research in osteoporosis: Where are we? Where should we go next?

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Abstract

Fractures resulting from low bone mass and excessive skeletal fragility (osteoporosis) are common worldwide both in males and females, particularly in later years of life. Both fractures, and the most important predictor of fractures, bone mass, are now known to be strongly heritable. This fact, plus the current growth in genetic science, has led to a surge of genetic research in osteoporosis, mostly in the search for genes and their polymorphisms that are responsible for variation in bone mass. Finding the genetic basis underlying variation in bone mass will lead us to deeper understanding of the biology of bone mass accumulation, maintenance and adaptation to load. This, plus finding the genetic basis for overall variation in fracture risk per se, will facilitate the development of interventions, both pharmaceutical and non-pharmaceutical, to prevent and/or treat osteoporosis successfully. This research has produced a rather large number of gene loci that seem to influence bone mass. The challenge now is to refine the statistical genetics and the phenotypes involved so that we can confidently identify those gene loci that truly influence bone mass, and to find ways to study the genetic basis for the most direct disease outcome of interest, fracture.

Keywords: Genetics, Osteoporosis, Bone Mineral Density, Heritability, Fractures, Phenotypes

Introduction

".....Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture"¹. This definition has been widely accepted, although there is room for further refinement that will likely take place as time passes. Every one seems to agree that the only important manifestation of the excess fragility of the skeleton is fracture of any part of the skeleton, and that the risk of fracture increases with increasing age. The most important fracture sites are the spine, hip and wrist due their prominence in terms of incidence, morbidity, mortality and economic cost². The economic cost of caring for fractures is substantial², and increasing. The indirect costs may be even greater, considering the lost produc-

tivity and cost of long-term care. Most countries are recognizing this and seeking ways to lesson the impact on their societies through research aimed at improving the effectiveness of prevention and treatment interventions. As with all diseases, the most efficient means of doing this is through discovery of the basic pathophysiology of the disease process. This has led to a surge of research into the genetic basis of excess skeletal fragility now that we realize that there is a strong genetic component to it.

Bone mass measurement

Prior to the 1980's the dominant scientific paradigm for osteoporosis was that the pathogenesis of skeletal fragility was reduced bone mass. There was considerable radiographic and pathologic evidence supporting the case, and investigators believed that if we could only measure bone mass accurately and precisely in living humans we might be able to solve the problem, or at least come a long way toward its solution. In the 1980's we became able to do this, first with single photon absorptiometry (SPA), then dual photon absorptiometry (DPA) and now dual energy x-ray absorptiometry (DXA). While other methodology is important (quantitative computed tomography, QCT), DXA measure-

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<u># of Genes</u>	<u># of Studies</u>
Major Candidate Genes, Found Associated with BMD	
32	32
Association Studies with Positive Findings (BMD)	
45	202
Association Studies with Negative Findings (BMD)	
13	88
*Various BMD Phenotypes, taken from reference (12)	

<u># of Loci</u>	<u># of Markers</u>	<u># of Candidate Genes</u>
55	48	44**
*Various BMD Phenotypes, taken from reference (12)		
**Identified from the linked regions, others may be present.		

ments now constitute a principal component of our study of osteoporosis, both as bone mineral content (BMC), an expression of the mass of the entire skeleton or any locus, and bone mineral density (BMD), an expression of the mass per unit area of silhouette of the entire skeleton or any locus.

Heritability

It has been known for some time that osteoporotic fractures tend to occur in families, and a family history of the osteoporotic fracture syndrome is regarded as a risk factor for fracture for an individual^{3,4}. However, more sophisticated recent studies have documented that the most important surrogate for fracture, bone mineral density (BMD), is strongly heritable at every skeletal site^{3,5}. Heritability is defined as the proportion of phenotypic variation that is attributable to genetic components. Usually expressed as h^2 , it is in the range of 50-80%^{6,7}, meaning that 50-80% of the variation in BMD is inherited. While these studies probably overestimate heritability because of inability to adjust accurately for similarities in the developmental environment of kindreds, the heritability for bone mass and density measurements is, nevertheless, accepted as quite strong.

Why bother with genetic studies in osteoporosis?

There are several reasons that justify our efforts at finding the genetic basis of skeletal fragility, such as to identify susceptible populations, or to customize treatments. However, the most important one is to investigate the basic biology involved in achieving and maintaining optimal skeletal strength and mass. Understanding the biology of bone's function as an adaptive, load-bearing organ would seem to

be the surest way to discover how to reverse or prevent skeletal fragility. A few genetic studies, such as recent ones from our laboratory and others on the phenotypes resulting from mutations in the gene coding for the lipoprotein related receptor-5 (Lrp5)⁸ illustrate this possibility. However, while fracture and bone density are strongly heritable, genetic study is made difficult by the fact that they are polygenic⁹, meaning that an unknown number of functional genetic polymorphisms are responsible for the variation we see in risk of fracture, or in surrogates for fracture, such as BMD. Nevertheless, these studies must carry on undaunted. They are turning out to be very expensive and resource intensive.

Where are we?

Linkage and Association Studies

Tables 1 and 2 are adapted from a recent survey of results of studies searching for the genetic basis of osteoporosis¹⁰. These studies used various bone mass (BMD) measurements as phenotypes, with the assumption that they are the major, even if not the only, determinant of fracture. Fracture itself is usually not the phenotype of choice for genetic studies of osteoporosis, although this is a strong consideration (see below). Given the large number of papers published in the last 6-8 years, it is obvious that there has been an upsurge of research activity in bone genetics. However, one can be left confused by the large number of association and linkage studies reporting positive identification of chromosomal loci or gene polymorphisms that are responsible for the heritability of BMD variation. Further, a number of loci or candidate genes test positive in some studies, and negative in others. This only leads to further confusion in the literature, and does

not help solve the problem of identifying those loci and genes that really do influence the BMD phenotype. Thus the search for genes responsible for determining variation in BMD is in a state of uncertainty or downright confusion at present.

What might be the explanation for this? The first that comes to mind for linkage analyses is that if all the large number of positive genome linkage sites were truly positive, then on average, each of them can explain no more than about 1-5% of the variation in the BMD phenotype. If that were the case, then none of the linkage studies reported for whole genome scans is sufficiently powered to detect them with confidence. Thus, while some of the loci may be true positives, most of them must be false positives that were identified in the linkage analysis by chance. Discerning true positives from false negatives requires large-scale linkage mapping and fine mapping studies that have efficient designs and sufficient statistical *power*. Almost all current linkage studies in human bone genetics are underpowered. Powerful cooperative fine mapping and exclusion analyses may offer an alternative way to plough through the genomic regions that have been found.

It seems highly likely that the number of reported chromosomal loci truly affecting BMD found by linkage and association studies exceed the true number by a considerable margin. Further, Deng et al.¹¹, have found evidence of the existence of one prominent locus (not yet identified) affecting variation in BMD measures.

Candidate gene association studies in populations have another problem that confounds the analysis and yields false positive results. It is the effect of unrecognized population admixture that confounds the association due to a subpopulation in the study cohort that contains higher or lower BMD values, and also, by chance, has a higher or lower distribution of an allele of a given marker or candidate gene. Thus, higher or lower BMD values in the entire study population can segregate by chance with the given candidate gene or marker. Further, positive findings in an association study may be due to linkage disequilibrium of BMD with another locus that is causally related to variation in the phenotype. In this situation, the association may be significant, but it is only due to the fact that the true locus responsible for the genetic variation in the phenotype is near enough to escape recombination often enough to account for the positive association.

Phenotypes

The commonest phenotype studied so far has been DXA measurements of BMD or BMC at any site, or the total body. The reasons for the choice are that it is a predictor of fracture, is easy to measure, and has strong heritability⁴. Ordinarily these measurements are adjusted for age, menopausal or gonadal status, environmental factors, gender, and other non-genetic factors. One can use the BMD Z-score as a surrogate phenotype for peak bone mass. This is the BMD or BMC value expressed in standard deviations of mean values from a reference population of broad age distribution. It also adjusts for gender since there are separate

reference populations for both genders that are installed in the commonly used DXA instruments. It is important to exclude subjects that have histories of illnesses, treatments or conditions that cause non-genetic variation in the DXA phenotype from populations selected for genetic studies.

While the BMD phenotypes have been the focus of nearly all the human gene association and linkage studies, there are strong reasons to consider alternatives. The paradigm for osteoporosis has been changing. We now understand that while DXA measurement is important, and is the most convenient method of predicting future risk of fracture, a low BMD value is not tantamount to "osteoporosis", or skeletal fragility. For example; change in BMD on treatment is not concordant with change in risk of fracture¹³, risk of fracture increases with age independent of change in BMD¹⁴, previous fracture is predictive of future fracture independent of BMD¹⁵, remodeling rates predict risk of fracture independent of BMD¹⁶, and while hip fracture and hip BMD are both heritable, the genetic correlation between them is not significant¹⁷. Thus, studies of the genetic basis of "osteoporosis" are complicated. It is, indeed, a complex trait that makes choosing a phenotype for genetic study somewhat difficult, and further, surrogates for fracture, such as BMD, are also complex traits, difficult to study.

One approach to circumvent the problems with using DXA measurements as the phenotype for osteoporosis is to study fracture *per se* as the phenotype. This removes any problem associated with the DXA surrogate. However, most fractures are difficult to use as phenotypes. For example, hip fractures occur at older ages in life when many of the members of the kindreds are not longer living, and the fractures are themselves associated with considerable mortality. Spine fractures also occur at older ages, and are a difficult phenotype because most of them are "silent", thus requiring large-scale radiographic screening in order to identify affected individuals to recruit to study. Other fractures may be infrequent enough that recruitment is difficult and expensive. However, one fracture that lends itself to genetic study is distal forearm fracture⁴. It has the advantages of low mortality and morbidity, ease of identification and relatively young age so that siblings, offspring and even parents are often available for study. Further, heritability of forearm fracture is about 25%, and it has been accepted as an osteoporotic fracture⁴.

Unusual bone phenotypes have been particularly useful in studies of the biology of bone mass regulation. The most prominent of these was the recent discovery of a kindred with very high bone mass (The HBM mutation) that had a dominant mutation in the gene coding for the Lrp5⁸. Loss of function mutations in this gene result in a phenotype that has been called osteoporosis pseudoglioma with juvenile onset severe osteoporosis and blindness¹⁸. These kindreds have led to a new area of research into the Wnt signaling pathway for regulation of bone mass, and responsiveness to mechanical loading. These mutations illustrate the value of studying rare and unusual mutations as an approach to learning the biology of bone mass regulation, and designing pharmaceutical

interventions based on that information. Surely there are more of these in populations, and one should be alert to their existence. The chance recognition of rare, but informative phenotypes is the most efficient way to learn about bone biology by genetic studies. In the case of the HBM mutation, a kindred of 39 members was enrolled in a whole genome search that resulted in identifying the *Lrp5* mutation^{8,19,20}. This contrasts with sample sizes at least 3000 for standard sib-pair studies even in order to detect QTLs with individual heritabilities over 15%.

Where should we go next?

The following list of suggestions is presented from the discussion above. It focuses on human studies. It is surely not complete, but will give a viewpoint at this time point in the ongoing search for genes underlying variation in risk of osteoporosis.

1. We should continue our human study efforts to identify gene polymorphisms that are responsible for variation in bone mass, and variation in risk of fracture.
2. We should be studying all the relevant DXA phenotypes in whole genome searches. Further, we should be performing studies of the genetic basis of fracture *per se*. We should adjust the phenotype data for confounding variables such as gender, age, height, weight, lifestyle factors and other non-genetic influences on bone mass.
3. We need adequate statistical power for our whole genome scanning studies that will yield more robust statistics. We need help from our statistical geneticist colleagues in determining the proper sample sizes for robust statistics for the various analytical designs.
4. We need more efficient study designs so that sample sizes for whole genome searches are smaller and less expensive to recruit. An example is the Transmission Dissociation Test (TDT) that can yield robust statistics with sample sizes that are a relatively small fraction of the sample sizes now used in the standard whole genome scans used in linkage analysis.
5. We need to encourage more collaboration in recruiting large sample sizes. It is quite difficult for a single center to recruit samples sizes in the range of 3000 or more in order to perform robust linkage analysis. Pooling of recruitment from several centers is the obvious solution to allow expansion of sample sizes.
6. We need to be careful in designing association studies so as to avoid misleading results due to population admixture and linkage disequilibrium.
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