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The genetics of osteoporosis

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Abstract

Introduction: Osteoporosis is the commonest metabolic bone disease worldwide. The clinical hallmark of osteoporosis is low trauma fracture, with the most devastating being hip fracture, resulting in significant effects on both morbidity and mortality.

Sources of data: Data for this review have been gathered from the published literature and from a range of web resources.

Areas of agreement: Genome-wide association studies in the field of osteoporosis have led to the identification of a number of loci associated with both bone mineral density and fracture risk and further increased our understanding of disease.

Areas of controversy: The early strategies for mapping osteoporosis disease genes reported only isolated associations, with replication in independent cohorts proving difficult. Neither candidate gene or linkage studies showed association at genome-wide level of significance.

Growing points: The advent of massive parallel sequencing technologies has proved extremely successful in mapping monogenic diseases and thus leading to the utilization of this new technology in complex disease genetics.

Areas timely for developing research: The identification of novel genes and pathways will potentially lead to the identification of novel therapeutic options for patients with osteoporosis.

Key words: osteoporosis, genetics, gene mapping, genome-wide association studies, next-generation sequencing

Introduction

In just two decades, the genetics revolution has transformed our understanding of human disease. In a few years, the cost of whole-genome sequencing (WGS) will fall below $US1000 per genome, an unthinkable concept 5 years ago, and personalized health care informed by individual genetic profiling will become part of routine clinical practice. Exactly how this information will be used for diagnosis, risk prediction and pharmacogenomics at the point of healthcare delivery remains unclear—however, we are undoubtedly witnessing an unprecedented shift in the practice of medicine.

It is timely, therefore, to review the genetics of osteoporosis, one of the commonest and most costly of diseases worldwide, and to discuss future directions in research aimed at dissecting the cause of this disabling disease. Enormous progress has been made in mapping the genes responsible for osteoporosis over the last 5 years, primarily through the development and implementation of genome-wide association studies (GWAS). Whether a similarly huge leap forwards is seen with the latest technological advancement of massive parallel sequencing remains to be seen.

Clinical background

Osteoporosis is the commonest metabolic bone disease worldwide. The hallmark of osteoporosis is low trauma fracture, and nearly half of all women and a quarter of all men over the age of 60 will suffer an osteoporotic fracture in their remaining lifetime.[1] The most devastating of osteoporotic fractures is a hip fracture, after which 50% of patients fail to regain their pre-fracture mobility, 25% end up in long-term residential care and 25% die within 12 months (see *Web Resources* below). It is not surprising, therefore, that the economic burden of osteoporosis is considerable, with recent estimates in the UK, Australia and the USA, projecting the annual costs associated with osteoporosis to be £2 billion (hip fracture alone), $AUS7 billion and $US17 billion, respectively.[2–4]

The strongest predictors of a future osteoporotic fracture are age, a previous fracture and low bone mineral density (BMD).[5] However, osteoporosis is also a familial disease. Children of individuals with an osteoporotic fracture are more likely to have low BMD themselves;[6,7] and the risk of low BMD for a sibling (of either gender) of someone with low BMD is six times that of the general population.[8] The genetic contribution to a disease is referred to as heritability (the proportion of the total variance of a trait that is determined by genetic factors). BMD is a highly heritable trait: 60–90% of BMD variation in the population is genetically determined (reviewed in Ref. 9). Significant heritability has also been observed in osteoporosis-related traits other than BMD, such as fracture risk, bone turnover rate and bone geometry, although the heritability of fracture risk appears less than that of BMD and may decrease with age. This is not surprising, as environmental influences on BMD also increase with age. Further, many if not most fractures result from falls; and falls risk is a complex trait with a large environmental contribution. However, it is worth emphasizing that all known loci associated with fracture are also associated with BMD,[10–12] supporting the relevance of BMD as a phenotype to understand the genetics of osteoporosis.

Mapping disease-causing genes

Like many other common diseases, osteoporosis is a complex disease genetically—many genes, each of small effect, contribute to the overall phenotype.[8] Efforts to map these genes originally focussed on candidate gene association studies and progressed to both candidate gene and whole-genome linkage studies. Despite many studies, very little progress had been made in mapping genes for osteoporosis (and most other complex diseases) by the turn of the century,[13] and it was hard to argue with the conclusions of one writer that 'the new genetics begins to appear like a relentless catalogue of failed aspirations'.[14] However, mapping genes for complex diseases was revolutionized by the methodological breakthrough of hypothesis-free GWAS.[15] Currently, over 60 genes are known to be associated with BMD at genome-wide significance (recently reviewed in Refs 16 and 17). Our inclusion of both current and previous methodologies is to provide the reader with an understanding of all of the approaches used in gene mapping for osteoporosis, including their strengths and weaknesses.

Candidate gene association studies

Association studies look at the frequency of a particular factor (whatever that may be—from smoking to living under electrical transmission wires to consuming vitamins, etc.) in cases compared with controls. In genetics, the factor examined is the rate of carriage of a particular genetic variant (or allele) in cases compared with controls. Candidate gene association studies select such variants in or near a gene of interest, based on previous literature suggesting its relevance to the disease in question. Although many candidate gene association studies were undertaken in osteoporosis, few genes were identified robustly, and the overall success of this approach was poor, as was the case for most complex diseases. Reasons for this poor performance include lack of statistical power to detect the small individual genetic effects acting in polygenic diseases, population stratification (differences in variant frequency between groups in the general population), differing linkage disequilibrium between a marker variant and the true disease-causing variants in different populations (see below for further discussion of linkage disequilibrium) and differing gene–environment and gene–gene interactions in different populations.[18] Further, interpreting the results requires a Bayesian approach—acknowledging that to accept a result as a true positive one requires overwhelming evidence of association, given the size of the genome and the prior probability of any particular variant being associated was remote—an argument often ignored. In a large study involving 19 000 individuals, Richards *et al.* assessed 36 000 single-nucleotide polymorphisms (SNPs) in 150 candidate genes chosen based on at least one previous study of this gene in osteoporosis. Only nine genes (*ESR1, LRP4, ITGA1, LRP5, SOST, SPP1, TNFRSF11A, TNFRSF11B* and *TNFSF11*) showed robust evidence of association with BMD at either femoral neck or lumbar spine, and a further four genes (*SPP1, SOST, LRP5* and *TNFRSF11A*) were associated with fracture risk, although this was independent of BMD (at least in part) only at *SPP1* and *SOST*.[19] At this point in time, we would argue that unless a candidate gene association study has levels of significance approaching genome-wide significance and is replicated in an independent population and/or has significant functional work, such studies should be regarded as hypothesis generating only. A further criticism of candidate gene studies is that, by definition, they cannot uncover novel pathways connected with disease, and so their utility in advancing the field is limited.

A special mention needs to be made of the vitamin D receptor gene (*VDR*), as undoubtedly the most studied gene in osteoporosis. The first published association study of variants in *VDR* and BMD suggested that 80% of variability in BMD was due to variants in this gene[20]—a result that was not really biologically plausible based on the observed inheritance of osteoporosis. However, this publication literally launched a thousand further such studies. To date, a definitive association of VDR with BMD or fracture has not been established robustly at genome-wide significance.

Given the important role of gonadal hormones in bone health, both in accrual and maintenance of bone mass, it is not surprising that *ESR1* (encoding the oestrogen receptor α) was also studied extensively in the pre-GWAS era—again, with conflicting results. Several GWAS have shown association of the region containing *ESR1* with BMD, although the exact variant responsible for the association signal may not ultimately be attributable to *ESR1* and it is possible that more than one association signal is present as associated SNPs from different GWAS are not in linkage disequilibrium with each other.[21,22]

Linkage

Linkage is a powerful approach for identifying mutations causing classical Mendelian, monogenic disorders. Generally speaking, mutations causing Mendelian disease are both rare in the general population, and highly penetrant, with an obvious effect upon phenotype. Linkage of a locus with disease is evident when genetic markers at or near that locus are inherited together (co-segregate) with disease phenotype within families. Linkage analysis was very successful in identifying the causative gene for many monogenic diseases.

In contrast to the success in mapping monogenic disease, linkage was not nearly so successful in

mapping polygenic diseases such as osteoporosis.[13] There are many reasons for this, including, again, a lack of power—a huge number of families would be needed for adequate power to detect the likely small effects of each individual quantitative locus affecting BMD.[23] Further, for an age-related disease such as osteoporosis, the penetrance of genetic risk factors may only become evident with age.

Both candidate gene and whole-genome linkage scans were undertaken in osteoporosis, primarily focussing on BMD although some included femoral neck geometry, ultrasound properties of bone and bone loss (reviewed in Ref. 9). However, even the largest study, a meta-analysis involving 11 842 individuals failed to demonstrate linkage with BMD at any locus at a genome-wide level of significance.[24]

Genome-wide association studies

In 1996, a prophetic paper was published, entitled 'The future of genetic studies of complex human diseases'.[25] The authors argued—incontrovertibly—that linkage was underpowered to identify the small to moderate genetic effects likely to be acting in common complex diseases (such as osteoporosis), and that a more powerful approach would be linkage disequilibrium mapping to perform large-scale GWAS. A major advantage was that such an approach did not require families but instead could use unrelated cases and controls. They also suggested that the appropriate level for statistical significance for a study of a million polymorphic markers would be $P < 5 \times 10^{-8}$.

At the time, such a study was, to a large extent, a theoretical experiment only. But in the following decade, advances in high-throughput genotyping technology (including optics and chemistry), study design and improved statistical analysis led to the development of GWAS and a revolution in modern disease genetics. Briefly, the technological advances mean that hundreds of thousands of variants (SNPs) dispersed throughout the genome can be genotyped simultaneously. Genotyping is undertaken in both cases and controls and the results analysed for evidence of association (differential genotype frequencies in cases compared with controls—see above discussion).

The proof that this approach could work was provided by the landmark GWAS paper published by the Wellcome Trust Case Control Consortium.[15] Suddenly, 24 loci were identified with association for seven major diseases, at genome-wide level of significance, with a further 58 probable loci (most of which were subsequently verified). The use of GWAS has meant that there is now robust evidence of association for a vast range of common complex diseases, with over 2000 loci identified for human diseases at *P*-value of $<5 \times 10^{-8}$ (see *Web Resources* below). This has had enormous impact upon our understanding of pathogenesis for almost all common human diseases, which may lead to the development of novel risk prediction and diagnostic strategies, and highlight potential therapeutic developments.

GWAS exploit linkage disequilibrium (LD)—where SNPs are inherited together more often than they should be by chance (i.e. 50% of the time, as predicted to Mendel's law of random assortment). This occurs because SNPs lying physically close to each other on a chromosomal strand are unlikely to be separated at meiosis: instead, they are inherited together on that chromosomal strand (known as a haplotype). The extent of LD and hence haplotypic structure in the genome has been determined through large mapping projects such as the HapMap project (see *Web Resources* below). The immediate applicability of knowing the haplotypic structure is that one can infer the genotypes of all SNPs on a shared haplotype block through genotyping of only a single SNP—this SNP effectively 'tags' the entire haplotype block. Thus, by genotyping only a relatively small number of SNPs, one can impute the genotype of a much greater number of variants, all of which can then be assessed for association in the trait under question. This approach has allowed meta-analysis of studies genotyped by different platforms—even if only a small fraction of SNPs are genotyped by both studies, the genotypes of many other SNPs can be imputed allowing for a much larger group of overlapping SNPs for association analysis.

Several large GWAS have been undertaken in the field of osteoporosis genetics, resulting in an explosion of BMD-associated genes (reviewed in Refs 9, 16 and 17). More recently several meta-analyses have been

employed combining data from previously published smaller studies to enhance sample size, with consequent increase in statistical power and new gene discovery. The largest osteoporosis meta-analysis included data from collaborators from 17 GWAS encompassing 33 000 individuals of European and East Asian ancestry, with replication in over 100 000 independent subjects. This study confirmed the association of 24 pre-existing genetic loci and identified a further 32 novel associated loci with BMD; 14 loci were also associated with fracture risk.[10]

GWAS results can be further explored using advanced data mining algorithms. For example, the Gene Relationships Across Implicated Loci (GRAIL) algorithm can elucidate further genes associated with known biological pathways and identify new connections.[26] GRAIL analysis in the GWAS meta-analysis published by Estrada *et al.* showed that the identified genes cluster in pathways: WNT/β-Catenin; RANK-RANKL-OPG and endochondral ossification (see following sections for further discussion of these pathways). These pathways were not novel discoveries heralded by GWAS results; however, their identification validates GWAS as a means of identifying pathways of relevance to the biological system under examination. Further, several of these pathways are already exploited as therapeutic targets in osteoporosis (e.g. the use of denosumab, targeting the RANK-RANKL-OPG pathway). This suggests that exploiting the therapeutic potential of other pathways identified through GWAS of BMD and osteoporosis will similarly lead to effective new agents for fracture prevention.

Current knowledge in osteoporosis

Collectively, over 60 loci have been associated with BMD with 15 loci associated with fracture risk (reviewed in Refs 16 and 17). It is beyond the scope of this article to review in depth the putative biological roles for these many genes; however, we will discuss the three pathways identified by GRAIL analysis. We presented above a discussion of two candidate genes of note (*VDR* and *ESR1*)—though would note here that only *ESR1* has evidence from GWAS of a significant role in population BMD variance.

WNT/β-catenin pathway

The WNT signalling pathway is essential for the development of many systems during embryogenesis. In bone, this pathway plays critical roles in skeletal development, limb patterning, bone mass accrual and maintenance, and fracture repair. The wnt proteins are a family of secreted glycoproteins involved in multiple signalling cascades; the best known and characterized being the WNT/β-catenin (or canonical WNT) pathway. This pathway is activated when wnt proteins form complexes with membrane-spanning frizzled receptor proteins and low-density lipoprotein receptors (LRP5 and LRP6), resulting in the stabilization of β-catenin. The stabilized β-catenin then translocates to the nucleus, where it controls the expression of target genes. SOST, secreted by osteocytes, inhibits signalling through this pathway and thus inhibits osteoblastic synthesis of new bone (recently reviewed in Ref. 27).

Genes in this pathway were among the first to be identified in osteoporosis GWAS, starting with *LRP5*,[28] with multiple other genes also identified at genome-wide significance [*AXIN1, CTNNB1, DKK1, GPR177, JAG1, LRP4, LRP5, MEF2C, RSPO3, SFRP4, SNT16, SOST, WNT4, WNT5B* and *WNT16* (reviewed in Ref. 17)]. *LRP5* is one of very few genes showing association in candidate gene association studies[29,30] subsequently validated in the GWAS era. *LRP5* mutations have been identified as the cause of both low bone mass and high bone mass skeletal dysplasias—respectively, osteoporosis pseudoglioma syndrome (MIM 259770) and a high bone mass phenotype (MIM: 601884). Mutations in SOST cause a high bone mass phenotype of van Buchem's diseases (MIM: 607636) and sclerosteosis (MIM: 269500). Of note, the use of anti-SOST antibodies in osteoporosis is now in Phase 3 clinical trial.

RANK-RANKL-OPG pathway

RANK, RANKL and OPG are members of the tumour necrosis factor (TNF)-related transmembrane cytokine superfamily, encoded by the *TNFRSF11A*, *TNFRSF11* and *TNFRSF11B* genes, respectively. RANKL, produced by osteoblasts, binds to RANK on

osteoclasts resulting in osteoclast recruitment, differentiation and activation. Osteoprotegrin (OPG), also produced by osteoblasts, is a soluble decoy receptor that blocks the binding of RANKL to RANK, preventing bone resorption (reviewed in Ref. 31).

This pathway was also one of the earliest to be associated with BMD and fracture risk in the population, with OPG, RANKL and RANK all detected in the first two comprehensive GWAS published in osteoporosis[21,28] and subsequently confirmed by many independent GWAS and meta-analyses.[10,11,22] Mutations in *TNFRSF11* (RANKL), *TNFRSF11A* (RANK) and *TNFRSF11B* (OPG) genes also have been identified in several skeletal dysplasias, including early onset Paget's disease (MIM 602080) and familial expansile osteolysis (MIM 174810), demonstrating their importance in bone physiology. A monoclonal antibody against RANKL, denosumab, inhibits RANKL signalling through RANK and subsequent osteoclast stimulation and is now widely used in osteoporosis treatment.

Endochondral ossification pathway

The majority of bones in the human skeleton form through the process of endochondral ossification, whereby osteoblasts deposit bone matrix (both collagen and non-collagenous proteins) on a cartilaginous template, which subsequently mineralizes. Both Runx2 and Osterix (encoded by *SP7*) are important transcription factors in osteoblast differentiation from mesenchymal stem cells. Runx2 is involved in the differentiation of preosteoblasts, whereas Osterix (Osx) is essential for the downstream commitment of preosteoblastic cell differentiation into mature osteoblasts. GWAS have identified several key genes of this pathway associated with BMD and fracture including genes involved in the development of cartilage, cartilage ossification and osteoblast differentiation (including *IBSP, PTHLH, RUNX2, SOX6, SOX9, SPP1* and *SP7*). Knock-out (KO) mice studies of Runx2 and Osx show reduced bone strength (*Runx2* KO)[32] or a complete lack of mineralized bone (*Osx* KO).[33] Further, mutations in *RUNX2* cause cleidocranial dysplasia (MIM 119600) which arises from defective osteoblast differentiation with resultant impaired bone formation.

The future in osteoporosis genetics

Limitations of GWAS

Despite the unarguable huge breakthroughs of GWAS, only a small proportion of the total heritability of most complex diseases studied has been explained to date—the question of 'the missing heritability'.[34] Certainly in osteoporosis, only 5% of the heritability of BMD has been explained to date.[10] Some groups have demonstrated that a greater proportion of overall heritability may still be explained by common variants than previously thought.[35] Common variants truly associated with disease may fail to reach the stringent $P < 5 \times 10^{-8}$ threshold due to their very small individual contribution to the overall phenotype and insufficient sample size for adequate power to detect association. Means to discover this include new analysis approaches, GRAIL and other pathway analyses, or simply increasing sample size (the 'bigger is better' approach).[36]

An alternative possibility is whether the 'missing heritability' might be due to unmapped rare variants with modest-high effect upon a trait, which may be poorly captured by tag-SNP GWAS approaches. Tag-SNP approaches would only capture such rare variants if there were linkage disequilibrium between the rare variant(s) and the genotyped common variants. If there were no LD, then common variant tag-SNP mapping approaches (viz. GWAS) would not detect signal driven by the rare variant. Even for loci mapped through common variants, a contentious question is whether such loci harbour rare or low-frequency variants that are driving that association.[37] This has been observed in some common diseases (e.g. type 1 diabetes) where rare variants were found to have greater impact upon the trait than common variants in the same genes.[38] However, other authors have challenged this viewpoint suggesting that most GWAS association are not driven by rare variants.[39]

Certainly in bone disease, the existence of rare mutations with large effect upon phenotype is evident from the many mapped skeletal dysplasias, including those with both high and low BMD.[40] At least some of these loci also harbour variants that contribute to population BMD variance, many

of which have already been mentioned above; other examples include *GALNT3*, *CLCN7* and *LTBP3* associated both with population variation in BMD[11] and with individual skeletal dysplasias (tumoural calcinosis [MIM:211900], osteopetrosis [MIM: 166600 & 611490] and tooth agenesis [MIM:613097]).

The actual causal variant or gene underlying the GWAS association may not be in either the associated SNP or obvious candidate gene, as loci may contain more than one plausible gene. Many GWAS hits occur in gene deserts, suggesting a major role of non-coding RNA in determining disease traits. Although non-coding-associated variants may be acting upon adjacent genes, this assumption may not hold true. Recently, this was demonstrated for the genetics of obesity, where the putative associated gene (FTO) proved to be completely innocent: the observed signal was due to long-range functional connections with IRX3.[41] Further mapping is usually needed to narrow down the exact gene and variant driving the observed association, either with further targeted genotyping (e.g. the 'Immunochip'[42]) or with targeted sequencing of identified loci.

Strategies to progress gene mapping in common diseases

Several strategies can be employed to improve mapping of genes for common diseases such as osteoporosis.[43] Firstly, and most simply, the 'bigger is better' approach undoubtedly improves gene identification[36] as has proved demonstrably successful in the osteoporosis field.[10] Secondly, one could use 'smarter approaches', such as the use of extreme cohorts that may be enriched in variants with greater individual effect on phenotype. Although the latter point remains to be proved, it is certainly true that the use of an extreme cohort increased power to detect common variants contributing to BMD.[11] Other 'smarter' approaches might also include the use of genetic isolates and/or transethnic mapping. Lastly, one could employ 'deeper approaches'. These could include deep replication of discovery GWAS findings —for example, using Metabochip or Immunochip or other further LD mapping. The latest advances in genetic technology – massive parallel sequencing—allow for targeted sequencing of existing loci (identified through common variant association mapping) to find the causative variant driving the observed association.[38] Further, massive parallel sequencing will detect low frequency and rare variants representing new gene associations for the disease under consideration.

Massive parallel technologies—including whole exome sequencing and WGS—have been extremely successful in mapping monogenic conditions, even with extremely limited clinical data.[44] Increasingly, they are being employed in cancer genetics, with the long-term aim of targeting chemotherapy according to tumour genotype. However, whether massive parallel sequencing will result in mapping new loci for common diseases is as yet uncertain. Early studies have been somewhat disappointing, with no new loci identified that were not already known from common variant mapping in celiac disease and thyroid disease.[45]

So from a practical viewpoint, what are the clinical implications of our knowledge of the genetics of osteoporosis at this point in the story? It would be honest to say that current fracture risk prediction from assessing carriage of known risk alleles for low BMD does not improve fracture prediction beyond the clinical risk factors of age and BMD scanning.[10] This is consistent with data from other studies, with a recent suggestion that most diseases will not prove predictable by genetic testing (with possible exceptions of type 1 diabetes, Alzheimer's disease and male coronary heart disease).[46] Nor is there yet evidence for a pharmacogenomic approach to managing osteoporosis—either for making the most effective therapeutic choice for a particular patient or for avoidance of side effects. However, there is evidence that new therapeutics pathways have already been identified (e.g. the role of the Wnt16 pathway in bone) that may lead to novel therapeutic options for patients with osteoporosis. Further, to paraphrase former United States Secretary of Defence, Donald Rumsfeld, we do not yet know what we do not know: massive parallel sequencing may yet lead to new breakthroughs in the genetics of osteoporosis.

Web resources

http://www.genome.gov/sequencingcosts/ (accessed 3 July 2014). HapMap: http://hapmap.ncbi.nlm.nih.gov/; Online Mendelian Inheritance in Man: http://www.omim.org/; http://www.2million2many.org/files/2 m2 m/public/content/file/38/upload/11.pdf (accessed 3 July 2014). GWAS-significant hit catalogue: www.genome.gov/GWAStudies).

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Conflict of Interest statement

The authors have no potential conflict of interest.

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