Science, medicine, and the future

Osteoporosis

Stuart H Ralston

Fractures related to osteoporosis occur in about 150 000 people annually in the United Kingdom and account for over £750m in healthcare costs. As the age of the population increases, osteoporotic fractures will become even more common unless we can develop better methods of identifying people who are at high risk of the disease before fractures have occurred, or have developed more effective ways of reversing bone loss in patients with established osteoporosis.

In this article I review the importance of genetic factors in the pathogenesis of osteoporosis and discuss the molecular genetic approaches that are being used to define the genes involved. I also describe some recent advances in understanding the cellular and molecular basis of bone remodelling and suggest how this knowledge might impact on the development of new drug treatments for osteoporosis.

Genetics and osteoporosis

Osteoporosis is a complex disease that is influenced by environmental factors such as diet, smoking, alcohol intake, and exercise, but genetic factors are now recognised to be one of the most important determinants of bone mass and risk of osteoporotic fracture. This knowledge comes from studies of bone mineral density in twins and in families, which have suggested that 70-85% of the inter-individual variance in bone mass is genetically determined. Although the genes that regulate bone mass are incompletely defined, current data suggest that several genes, each with modest effects on bone mass, are involved, rather than one or two genes with major effects; however, the exact number of genes involved and their relative effects remain unclear.¹

Approaches to defining genetic contribution to osteoporosis

Two approaches are usually used to dissect out the genetic contribution to complex diseases such as osteoporosis.² The first is to look for evidence of allele sharing on a genome-wide basis by means of polymorphic genetic markers in sib pairs (a "genome search"), and the second is to look for evidence of an association between polymorphisms in candidate genes and osteoporosis in case-control or population studies. Each of these methods has advantages and drawbacks.

Studies of sib pairs have been used to analyse the genetic basis of complex diseases like asthma and diabetes, but they may be of less value in detecting the

Possible future developments

Defining distinct pathogenic subgroups of osteoporosis with different genetic and environmental influences

Use of genetic markers for predicting risk of osteoporosis

Defining new molecules to act as targets for drugs to prevent bone loss

Defining new molecules to act as targets for drugs to reverse established osteoporosis

genes involved in regulation of bone mass. This is because the statistical power of studies of allele sharing is critically dependent on the strength of the association between individual genes for susceptibility and the disease. Even though genetic influences on bone mass are strong overall, if it turns out that these influences result from an equal contribution from many genes, each with a small effect, it may prove impossible to detect these genes in a sib pair study unless the study is extremely large.³

In view of these problems, it is the second approach, of candidate gene analysis, that has proved most popular in studying osteoporosis.

Candidate gene studies

Candidate gene studies in osteoporosis are facilitated by the fact that a great deal is known about the factors that regulate bone turnover and the proteins that make up normal bone matrix. This gives us a wide range of potential candidate genes, but relatively few of these have been studied in humans so far (table 1).

The genes for collagen type I are important candidates since this is the major protein of bone and recent work has identified a polymorphism that alters a binding site for the transcriptional regulatory protein Sp1 in the COL1A1 gene (which encodes one of the collagen type I peptide chains).⁴ This polymorphism seems to be associated with low bone density and osteoporotic fracture in British women, and studies are in progress to define its clinical value in predicting osteoporotic fracture in other populations.

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Table 1 Some candidate genes for studying genetic contribution to osteoporosis

Gene studied	Rationale for study
Vitamin D receptor	Vitamin D regulates bone cell differentiation, bone turnover, and calcium homeostasis by interaction with vitamin D receptor
Oestrogen receptor	Oestrogen regulates bone turnover and skeletal growth by interaction with oestrogen receptor
Transforming growth factor β	This is present in bone matrix and is thought to couple bone resorption and bone formation
Interleukin 6	This regulates osteoclast differentiation and may mediate some actions of sex hormones in bone
Collagen type I genes	Type I collagen is the most abundant protein in bone
Collagenase	This is a degradative enzyme involved in resorption of bone matrix

Polymorphisms of the gene for the vitamin D receptor have also been studied in relation to bone mass.⁵ Those most extensively studied are recognised by restriction enzymes in the 3' region of the gene between exons 8 and 9. They do not cause changes in the vitamin D receptor protein, and their relation with bone mass have been poorly reproducible in different studies.⁵ Another polymorphism in exon 2 of the vitamin D receptor gene is of greater interest since it introduces an alternative translational start site, yielding a vitamin D receptor protein some three amino acids smaller than the normal vitamin D receptor,⁶ raising the possibility that these two variant proteins may differ in terms of function. The exon 2 polymorphism is associated with much greater genotype-specific differences in bone mineral density than the original vitamin D receptor polymorphisms and could turn out to be a better predictor of osteoporosis in clinical practice, but further studies will be required to confirm this.

Polymorphisms in other candidate genes such as those for interleukin 6, transforming growth factor β , and oestrogen receptor have also been found to relate to bone mass or osteoporotic fracture in some studies,⁷ but so far these observations have generally been confirmed in single populations only and more studies are necessary. As the human genome project moves forward, still more candidate genes will become available for analysis and it has been argued that systematic screening of these genes in association studies may represent the most efficient and powerful way of investigating the genetic basis of diseases like osteoporosis.³ Such studies will probably show that osteoporosis is not a single disease but, rather, is a single phenotype that results from inherited variation

in several genes which are involved in regulating bone cell growth and differentiation and matrix composition.

Clinical application of genetic studies in osteoporosis

A potential application of genetic studies in this subject is in assessing the risk of osteoporotic fracture. If we can identify polymorphisms that consistently predict low bone density or osteoporotic fracture, then these could be used as a screening tool in order to identify people at risk and guide the need for prophylactic treatment before fractures have occurred. It might also be possible to use genetic tests to predict who would respond best to anti-osteoporotic treatment. For example, it has already been shown that the response of bone mass to dietary supplementation with vitamin D and calcium is partly dependent on vitamin D receptor polymorphisms.⁸ Similar use of polymorphic markers in other candidate genes might be applied in the future to determine who would best respond to other treatments such as hormone replacement therapy, bisphosphonates, and modifications in lifestyle such as exercise.

Lessons from other bone diseases

Studies on the genetic basis of less common bone diseases such as osteopetrosis and Paget's disease may also help in understanding the pathogenesis of osteoporosis and in the search for new drugs to prevent and treat the disease. An example of this is provided by pycnodysostosis, a rare inherited form of osteopetrosis (abnormally dense bone) that has recently been discovered to be due to an inactivating mutation in the gene for cathepsin K.⁹ Cathepsin K is a protease that is highly expressed in osteoclasts and which is essential for the normal resorption of bone matrix. Since antagonists of cathepsin K inhibit bone resorption in vitro, cathepsin K inhibitors may represent a new class of antiresorptive drugs for future clinical use.

Cellular basis of bone remodelling

Bone remodelling is a coordinated process of cellular activity that is responsible for the renewal and repair of damaged bone throughout adult life. Understanding the cell biology of this process is important, since abnormalities of bone remodelling underlie virtually all metabolic bone diseases.

Methods based on cell culture have been devised to investigate the mechanisms by which bone cells are formed and activated and to study the mechanisms by which they communicate with each other during the remodelling process. These studies have shown that bone remodelling begins with recruitment of osteoclast precursors to the site that is to be remodelled, where they differentiate into mature osteoclasts (fig 1). Although the mechanisms that determine where and when remodelling occurs are unclear, it is probably triggered by mechanical stimuli or release of chemotactic factors from microfractures in damaged bone. During the phase of bone resorption, osteoclasts remove a specific amount of bone, and they then undergo programmed cell death (apoptosis) in the reversal phase. Several drugs and hormones that

Fig 1 Bone remodelling. Remodelling begins with attraction of osteoclasts to the target site, probably triggered by mechanical stimuli or release of chemotactic substances from areas of microdamage in bone. Mature osteoclasts remove bone during the phase of bone resorption. After resorption is complete, osteoclasts undergo apoptosis during the reversal phase, and bone formation begins as osteoblasts are recruited to the site. Some osteoblasts become trapped within bone matrix and differentiate to form osteocytes, which act as sensors of mechanical strain in bone

Generation of gene knockout mice

- Embryonic stem cells are injected with targeting DNA that contains an inactivating mutation in the gene of interest, along with the gene that codes for neomycin resistance
- The new DNA becomes incorporated into the host chromosome in place of the normal gene in some cells
- Cells that contain the mutated gene are selected for by culturing in medium containing neomycin (which is toxic to cells that do not contain the mutated gene)
- Cells bearing the mutated gene are injected into early mice embryos, yielding mice in which one copy of the target gene is inactivated
- These mice, each containing one copy of the mutated gene, are interbred to give "knockout mice" in which both copies of the gene are mutated
- If the knockout mice show abnormalities, this implies an essential function for the gene in affected tissues and organ systems

inhibit bone resorption, such as bisphosphonates and oestrogen, are now thought to act, in part, by promoting osteoclast apoptosis.¹⁰

Bone formation follows on from the reversal phase, beginning with recruitment of osteoblast precursors to the remodelling site. These cells then differentiate into mature osteoblasts and start to form new bone matrix (osteoid), which subsequently becomes calcified to form mature bone. Some osteoblasts become buried in the newly formed bone matrix to form osteocytes, which interconnect with each other and with lining cells on the bone surface. Recent data suggest that osteocytes probably act as mechanoreceptors in bone,¹¹ secreting small molecules such as prostaglandins and nitric oxide in response to mechanical stimulation, which then influence the function of other bone cells such as osteoclasts and osteoblasts.

Use of gene knockout mice in studying mechanisms of bone turnover

Over the past 10 years, studies in gene knockout mice (see box) have yielded important insights into the molecular mechanisms that underlie the differentiation and function of bone cells, and this technology will probably be increasingly used to define the role of candidate molecules in the regulation of bone turnover.

Mechanisms of bone resorption

It has long been established that osteoclasts derive from haemopoietic precursors, though the factors that regulate this process have, until recently, remained unclear. Gene knockout experiments have now defined some of the key molecules that regulate osteoclast differentiation and where they act in the process of osteoclastogenesis. Knockout of the haemopoietic transcription factor PU.1 results in the phenotype of osteopetrosis, which is characterised by defective osteoclast formation, and deficiency of tissue macrophages (fig 2). These data identify the PU.1 molecule as an important molecular "switch" which is essential for regulating the early stages of osteoclast/macrophage development.¹²

A similar phenotype occurs in a strain of mice that are naturally deficient in the myeloid growth factor M-CSF, which is normally produced by the stromal cells of bone marrow and which acts on haemopoietic cells to enhance development of osteoclasts and macrophages. In contrast, knockout of the transcription factor *c-fos* causes osteopetrosis with normal macrophage formation, placing this factor downstream of both PU.1 and M-CSF in osteoclastogenesis.¹³ The *c-src* gene acts further down still, since mice deficient in *c-src* are able to form osteoclasts, but these cannot resorb bone normally because they cannot form the ruffled border that is essential for resorption.¹⁴ Deficiency of other molecules such as cathepsin K, carbonic anhydrase II, and tartrate resistant acid phosphatase impair the ability of mature osteoclasts to resorb bone.

Although these studies may seem esoteric to doctors involved in the day to day treatment of metabolic bone diseases, identification of these molecules is an extremely important advance since they represent key targets for the development of future drugs to reduce bone resorption.

How bone cells talk to each other

Osteoblasts and bone marrow stromal cells also play an important role in regulating osteoclast formation and activity. Apart from releasing M-CSF, a key factor in osteoclast differentiation, it is now recognised that many of the factors that modulate bone resorption do not directly stimulate osteoclasts but, rather, trigger the release of substances from bone marrow stromal cells or osteoblasts, which then act on cells of the osteoclast lineage to modulate formation and activity of osteoclasts.¹⁵ Small molecules such as hydrogen ions, hydrogen peroxide, and nitric oxide¹⁶ have been identified as possible candidates for this activity, but other factors probably remain to be discovered.

Mechanisms of bone formation

Gene knockout studies have also started to define the mechanism by which the differentiation and function

Fig 2 Osteoclast development. Studies in knockout mice with osteopetrosis and in humans with rare inherited forms of osteopetrosis have revealed an essential role for several key proteins (italicised) in development and function of osteoclasts. Studies based on cell biology show that stromal cells and osteoblasts secrete substances such as macrophage colony stimulating factor (M-CSF), nitric oxide (NO), hydrogen peroxide (H₂O₂), and hydrogen ions (H⁺) that also influence osteoclast development and activity

Fig 3 Osteoblast differentiation. Studies in knockout mice have revealed an essential role for several key proteins (*italics*) in osteoblast differentiation and function. The CBFA1 transcription factor is an essential molecular switch for osteoblast differentiation, whereas the PPAR γ transcription factor (peroxisome proliferation activated receptor) acts as a switch for adipocyte development. Osteocalcin may act in an autocrine manner to inhibit osteoblast function

of osteoblasts are regulated. An important finding has been identification of the protein CBFA1 as an essential mediator of osteoblast differentiation (fig 3).¹⁷ CBFA1 is a transcription factor that binds to specific recognition sequences of DNA in the promoter region of several genes that are expressed in osteoblasts. Knockout of this factor completely prevents osteoblast differentiation, resulting in a phenotype in which the skeleton is composed of cartilage and fibrous tissue.¹⁷ Moreover, forced expression of the CBFA1 protein in non-osteoblastic cells causes differentiation into osteoblasts, suggesting that CBFA1 may represent the “master switch” that triggers osteoblast differentiation.

Related work has shown that knockout mice deficient in osteocalcin have increased bone mass due to increased bone formation.¹⁸ Although serum osteocalcin concentrations are generally thought to act as a marker of osteoblast activity, the gene knockout data imply that it has an important physiological function as an inhibitor of osteoblast activity.

The implications of these studies for treating osteoporosis are profound. Although we have several drugs that inhibit bone resorption, treatments that stimulate bone formation are much thinner on the ground. If it were possible, for example, to develop

drugs that could mimic the effects of CBFA1 we would probably have a powerful means of stimulating new bone formation, which could be clinically useful in treating established osteoporosis.

Conclusion

Advances in molecular genetics and cell biology have revolutionised our understanding of the basic mechanisms that underlie the regulation of bone remodelling and have shown the importance of genetic factors in the pathogenesis of osteoporosis and other bone diseases. Clinical studies have already shown associations between polymorphisms of several candidate genes and bone mass, and it is likely that many more candidates will be found. Definition of these genes is important in offering the prospect that patients at risk of osteoporosis may be identified by genetic testing before the disease has become established, which would allow better targeting of high risk individuals for prophylactic treatment.

Advances in the cell biology of bone and studies of transgenic mice have identified some of the key molecules that regulate the differentiation and function of bone resorbing and bone forming cells. This information, while seemingly far removed from clinical practice at present, will be of crucial importance in the development of new drugs for preventing and treating osteoporosis.

A human osteoclast

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