REVIEW

Genetic influences on bone turnover, bone density and fracture

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Fractures due to osteoporosis are an increasing healthcare problem in all ageing communities. A major determinant of fracture risk is bone density, independent of other contributory factors such as falls and ageing (1–4). Bone density in later life is contributed to by both peak bone mass achieved in early adulthood and subsequent bone loss. There is compelling evidence suggesting that both peak bone mass and its subsequent loss are determined by combinations of genetic and environmental factors. To identify the molecular genetic factors that underly the genetic determination of bone density remains a sizeable challenge with potential impact on both therapy and prevention of osteoporosis. This review will examine the evidence for genetic contributions to osteoporosis in light of recent advances in this area.

Genetic epidemiology in osteoporosis

Prior to the development of bone densitometry, studies of the genetics of osteoporosis were limited by the absence of a reliable quantitative measure of bone density. Fracture is an event related not only to bone density but also to age and other factors such as falls and muscle strength. Therefore, the use of fracture prevalence or incidence to explore the genetic epidemiology of osteoporosis is flawed. For this reason, the identification of a continuous variable that identifies risk of fracture was required to allow detailed assessment of the genetics of osteoporosis. Bone densitometry then opened new possibilities for assessment of the inheritance of osteoporosis risk by defining a phenotype, i.e. bone density, that is the predictor of the index event, i.e. fracture.

Family studies suggest a significant effect of genetic factors on peak bone mass. For example, using the early approach of metacarpal cortical bone thickness, parent–offspring correlations suggested that bone mass was largely determined genetically (5). Similarly, using quantitative computed tomography, bone mass was noted to be reduced in healthy young adult relatives of a heterogeneous population of osteoporotic patients compared to controls with no family history (6). Significant correlations have been observed between younger women (mean age 18.6 years) and their mothers, after adjusting for body mass index (7). Whilst these latter studies have examined mother–daughter correlations, genetic factors would be expected to be contributed from both parents. Indeed, significant correlations have been observed between mid-parent bone density and the bone density of offspring, with estimates of the variance attributable to genetic factors, after adjustment for life-style factors, ranging between 46 to 62% for different skeletal sites, including the spine and femoral neck (8). In a large study of white elderly men and women, a family history of osteoporosis in either parent was a significant but weak predictor of low bone density (9).

Family studies of age-related quantitative traits, such as bone density, are confounded by the age differences between parents and their children. Within-family correlations may also relate to similar environments rather than to similar genes, particularly in the stages of rapid skeletal development of childhood and adolescence. Overall, family studies are only supportive of heritable influences on bone density, particularly peak bone density, because this influence could be related, at least in part, to familial influences on unaccounted environmental factors such as physical activity and calcium intake. To explore further the heritability of bone density, studies in twins have provided the most convincing evidence of major genetic influences.

Twin studies of bone mass

To assess the relative contribution of genetic and environmental factors to the population variance in a continuous trait such as bone density, the classical twin study has been an invaluable design. In monozygotic (MZ) twins, in whom 100% of their genes are identical, any intrapair differences in a trait must be due to environmental factors. On the other hand, in dizygotic (DZ) twins, intrapair differences or variation can relate to both genetic and environmental differences. The aim, generally, of two studies is to compare the within-zygosity correlations and/or variances in MZ and DZ twins in order to examine the genetic contribution to phenotype variation.

Twin studies by a number of investigators now support the strong genetic effects on bone density at both peripheral and axial bone sites (10–14). Early twin studies assessed peripheral bone mass, e.g. metacarpal cortical bone thickness and single photon absorptiometry of radial mid-shaft (11, 14). For example, early studies using single photon absorptiometry to measure bone mass in twins found a
significantly larger intra-pair difference in DZ twins, consistent with significant genetic determination of bone mass (14). There have also been twin studies using the technique of metacarpal morphometry that have suggested a significant genetic effect, with estimates of the heritability between 0.7 and 0.8 (11). The term “heritability” is often used in describing the possible genetic contribution to the population variance in a trait.

With the availability of dual photon and dual energy x-ray absorptiometry to measure axial skeletal sites, a strong effect of genetic factors on bone density at these sites has been demonstrated. Pocock and co-workers (12) observed significant effects in the lumbar spine and femoral neck, explaining the majority of the population variance in bone density. Covariance analysis suggested that the factors that produced the genetic effect at one site were probably responsible for the effect at other sites, i.e. a single gene or set of genes was responsible for the genetic effect at all sites. The largest genetic influence was observed in the spine and Ward’s triangle in the proximal femur, sites comprised predominantly of trabecular bone. In this study, forearm bone mass showed a significant genetic effect only for premenopausal women. Interestingly, a subsequent study also found a significant genetic effect at the radius in adults but only in younger twins (less than 25 years age) at the spine (10).

Although twin studies are powerful tools to study genetic effects, they have some inherent limitations. While the twin design has been popular in medical research, inferences drawn from twin data are open to debate in both statistical and biological terms. In assessing the effect of zygosity on phenotype, twin studies can only imply rather than “prove” genetic influences. Twin studies assume in general that the environmental factors important in determining variability in a phenotype are not more similar between MZ and DZ twin pairs. If a simple non-additive genetic model is proposed, the correlation between identical twins (rMZ) should be approximately twice the correlation between non-identical twins (rDZ). A more highly shared environment in the MZ twins could increase the correlation between identical twins (rMZ) to more than double the correlation between non-identical twins (rDZ). In our study when bone density was analysed, controlling for known environmental confounders, the rMZ increased without having a major influence exerted on rDZ, suggesting that the overestimation of heritability, at least for the population studied in that particular series, may relate to complex gene environment or gene–gene interaction (15). Importantly, any estimate of heritability in one population is not necessarily “transferable” to other populations. Rather, until shown otherwise it must be considered as applicable only to the conditions and possible peculiarities of the population under study. For these reasons the “heritability index” is of relatively less importance than the observation that a trait is, overall, under genetic influence, i.e. the observation that a trait appears to be genetically influenced is more useful than quantification of the variance attributable to genetic factors.

Twin studies of bone density changes with age

Twin and family studies support a genetic effect on peak bone mass, but whether a genetic effect exists on rates of loss with ageing or after the menopause is as yet unclear. One set of observations in aging male twins did not suggest that changes in bone density at the distal radius were influenced genetically (16). Intra-pair correlation for the rate of change in distal radius over 16 years was no different for male MZ and DZ twins. However, the within-pair correlations for both twin types were significant, suggesting that factors common within twin pairs, such as common environmental influences may be important in determining age-related bone loss in these male twins, at least at the appendicular skeleton. As an example of possible shared environmental factors, accelerated rates of loss were seen in those men who smoked and consumed alcohol (17). In contrast, a recent study over a shorter (around 4 years) period in predominantly female twins suggested that rates of change at the trabecular sites of the lumbar spine and Ward’s triangle and influenced genetically (18). Interestingly, changes at the femoral neck did not appear to be influenced by genetic factors in this study. It is not yet clear whether the genetic effect is less strong at the more cortical sites or whether longer periods of observation are required to observe genetic effects on the more slowly evolving changes in bone density in cortical bone. Prospective studies in twins to address the effect of genetic factors on both the attainment of bone mass through adolescence and changes in the immediate postmenopausal period will be of considerable interest.

Twin studies of bone turnover

Biochemical parameters and markers of bone turnover appear to be under genetic control. Recent studies in twins suggest strong genetic effects on osteoblast function and bone turnover generally. In adult twins the majority of the population variance in serum osteocalcin, an osteoblast product, was determined by genetic factors (19). Importantly, in DZ twins, where intra-pair differences in serum osteocalcin levels may reflect genetic variance, differences in osteocalcin predicted within-pair differences in bone density at both the lumbar spine and femoral neck, with twins with the higher osteocalcin value being associated with the lower bone density (19). This is in accordance with other data indicating that lower bone turnover is associated with higher bone density (20).

Twin studies have also shown strong genetic effects
on markers of type I collagen metabolism (21). Because the predominant collagen in bone is type I collagen, specific measures of type I collagen turnover have been studied. A major part of the population variance in markers of both type I collagen synthesis and degradation can be explained by genetic factors (21). Interestingly, in the same study a marker of type III collagen synthesis was not found to be under genetic influence, suggesting that the genetic influence on type I collagen metabolism may reflect a specific effect on bone turnover rather than an overall influence on collagen metabolism per se. Also, as observed with osteocalcin, the twin with higher levels of these markers of collagen formation and degradation (i.e. higher bone turnover) had the lower bone density, especially at the spine. Interestingly, this effect tended to be independent of the influence of osteocalcin, further suggesting that the genetic regulation of collagen metabolism in bone may be independent of that regulating osteocalcin. Importantly, overall, these data support the hypothesis that the genetic influence on bone density is mediated through genetic regulation of bone modelling.

**Mechanisms of genetics effects on bone density**

Bone density is associated with muscle strength and physical fitness. Thus, one mechanism by which genetic factors could influence bone density is indirectly, via exercise and aerobic performance. Interestingly, in this regard, analyses by Seeman and colleagues of bone density and lean body mass in twins indicated that they may have common genetic determinants (22). Clearly, these possible common genetic influences warrant further detailed exploration in twin studies.

**Vitamin D receptor gene**

Vitamin D has a central role in regulation of bone and calcium homeostasis, regulating a network of genes in various tissues and cell types. The target genes for calcitriol action include key regulatory and structural genes in all the major sites of calcium homeostasis, including bone, gut, kidney and parathyroid glands. For example, the active hormonal form of vitamin D, calcitriol, acts on the osteocalcin gene via the vitamin D receptor (VDR). It has the capacity to act as a potent amplifier of gene regulatory signals. This function must be mediated in trans, because the osteocalcin and VDR genes are encoded on different chromosomes. The amplification process means that modest differences in the expression of the trans-acting factor (the VDR) could be expressed in large differences in the end product or expression of a target gene. Thus, because osteocalcin production and collagen synthesis are regulated by calcitriol through the VDR, genetic pathways involving vitamin D seemed likely candidates for the genetic effects on bone.

Based on these hypotheses, we examined the possible role of common allelic variation in the VDR gene to modulate bone and calcium homeostasis. We found that these alleles predict serum osteocalcin in normal women (23). Individuals whose alleles lack the Bsm-1 endonuclease cleavage site had significantly higher osteocalcin levels than those homozygous for the presence of the site (23). Owing to inherent difficulties in identifying multiple-generation and family studies for the assessment of linkage in osteoporosis, DZ twins have been used as a form of sib-pair analysis to explore linkage between these VDR gene alleles and bone density (Fig. 1). Such analyses support linkage between

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Fig. 1. The relationship between bone mineral density (BMD) and vitamin D receptor gene alleles in twins. The left panel describes the lumbar spine BMD in 22 pairs of non-identical twins plotted as twins vs co-twin, with the lines joining the twin pairs. Note that in 21 of the 22 pairs the twin with the B allele has the lower bone density. The right panel describes the lumbar spine BMD in all twins regardless of zygosity. Note again that the B allele is associated with lower bone density. (Copyright Nature, reproduced with permission.)
VDR gene alleles defined by Bsm-1 digestion and polymerase chain reaction (PCR) amplification, and the bone density phenotype (24). Dizygotic twins with the same VDR genotype had bone densities almost as similar as those of MZ twins, while those with different VDR allelotypes differed by up to 20% in bone density at spine and hip sites. Moreover, twins with the low bone density allele had lower bone density than their co-twin (Fig. 1). Also, VDR alleles contributed to the variance in bone density in a non-twin population (24). These data suggest that one particular VDR gene allele appears to determine higher bone turnover and lower bone density in both twin and unrelated populations. This model of assessment of the relationship between potential gene markers and bone density, by using DZ twins as sib pairs, will be useful in the assessment of other candidate genes that no doubt contribute to the genetic variation in bone density.

A number of other studies have now supported a relationship between VDR gene alleles and bone density in a variety of populations. Studying a group of postmenopausal twins, Spector et al. (25) also have found a relationship between VDR gene alleles and bone density in 192 DZ postmenopausal twins. They found that twins homozygous for the presence of the Taq endonuclease site (which is in strong linkage disequilibrium with the absence of the Bsm-1 site) had 10% lower bone density at both axial and appendicular sites than twins homozygous for the absence of the Taq site. Yamagata and colleagues (26) studied Bsm-1 VDR polymorphisms in 102 healthy unrelated Japanese women aged 23–68 years. They found that both a lower baseline lumbar spine bone density of around one standard deviation (BB vs bb) and a higher rate of loss in lumbar spine BMD in the BB group. The effect of the B allele appeared to be stronger in premenopausal women (N = 47) but the smaller sample size limits any conclusions in this regard. Interestingly, there are clear racial differences, with a lower frequency of the B allele in those Japanese women compared to Caucasians, observed by both Yagamata et al. and Tokita and colleagues (27). Importantly, Tokita et al. observed that the haplotype associated with absence of the Bsm-1 and Apa-1 sites was associated with higher calcitriol and lower bone density in Japanese premenopausal women (27). Shiraki et al. also observed a relationship between VDR allelic variation and bone density in Japanese women (28). A study from Austria (29), also in abstract, observed that vertebral trabecular density determined by quantitative computed tomography was related to VDR gene alleles. In a group of women from Rochester, Minnesota, USA, VDR genotype was a predictor of population variance of femoral neck but not lumbar spine bone density (30). In this population the bulk of the VDR allelic effect appeared to be on peak bone mass, with little effect evident in the elderly.

In contrast to the studies confirming a relationship between VDR gene alleles and bone density, Hustmeyer et al. (31) have reported data in a sample of North American twins failing to find a relationship. Using the same statistical analytical techniques employed by Morrison et al. they could not find a relationship between the Bsm-1 polymorphisms and lumbar spine bone density in healthy twins of a similar age group to Morrison et al. It is unclear why these results differ from those of Morrison et al. and Spector et al., but given the nature of the vitamin D endocrine system, environmental differences such as vitamin D and calcium intake may play a role, although this needs to be explored in more detail. In this regard, Ferrari et al. (32) in a prospective study examining calcium supplementation on bone density changes in the elderly found that the lumbar spine bone density response to calcium was related to VDR gene alleles, with those with the low bone density genotype having the greatest bone loss over 18 months. While environmental factors may confound observations of genetic effects on bone density, alternatively other polymorphic genes, as yet to be identified, may modulate the expression of the VDR gene effect in different populations, proposed by Parfitt (33).

Vitamin D receptor gene and bone density—what are the mechanisms?

Because the VDR allele effects on bone density correlate with osteocalcin level differences, and the osteocalcin and VDR genes are encoded on different chromosomes, the most likely mechanism of these effects is through a functionally different VDR, either in quantity or activity. Although it is possible that the VDR alleles are merely in linkage disequilibrium with the effect gene, this seems unlikely given the centrality of the vitamin D endocrine system to bone and calcium homeostasis and differences in the vitamin D endocrine system between individuals with the different BDR genotype (34). Preliminary data show differences in responsiveness to calcitriol in premenopausal women homozygous for different VDR alleles (34). The low bone density VDR genotype, as observed previously (24, 27), had significantly higher basal calcitriol, osteocalcin and phosphate levels compared to the higher bone density group, without any difference at baseline in intact PTH or serum total or ionized calcium. Following the administration of 2µg of calcitriol daily for 7 days, both allelic groups achieved similar serum levels of calcitriol and osteocalcin. However, significant differences in the response of serum phosphate, inorganic PTH and bone resorption indices were observed. To unravel the precise mechanism of these differences requires further work; however these data indicate differences in bone and calcium homeostatic pathways that relate to VDR gene alleles. However on the basis of current data one could suggest that the data suggest either a specific resistance to gut calcium absorption in the BB allelic group leading to elevated calcitriol levels,
Also, there may be a degree of resistance to the PTH suppressive effects of vitamin D. The price paid for efficient gut calcium absorption in this group may be elevated bone remodelling, which over many years may produce lower bone density. Clearly, further studies addressing these aspects of vitamin D and calcium endocrinology are required to examine these hypotheses in more detail.

The physiological correlates of the VDR gene alleles and the molecular mechanisms remain a challenge. The amplifying nature of trans-acting factors makes it possible that the relatively large differences observed in bone density, osteocalcin and other bone turnover marker levels could be due to very subtle differences in receptor level or function. The two major possibilities are a change in the coding region of the VDR gene, resulting in a functionally different receptor protein or, alternatively, a different level of otherwise identical receptor proteins. These possibilities are yet to be elucidated clearly.

Genetics and fractures

Bone density is now used to define osteoporosis. In this definition it is recognized that low bone density is necessary but not sufficient by itself for fracture to occur. Low bone density has been shown in several studies to be a fundamental factor in increasing fracture risk (1–4). Also, in prospective studies, a strong interaction exists between low bone density and postural instability fracture risk (4). Given the requirement for other events unrelated to bone density to the fracture event, it is not surprising that the small number of genetic studies of fracture risk have not been informative. In one retrospective case-controlled study, women of mean age 64 years whose mothers had sustained vertebral fractures three decades earlier had no increased frequency of fracture compared to an age-matched control group (35). However, the small sample size and retrospective nature may have been limiting.

Despite these limitations it remains an important question as to whether gene markers that may predict bone density may in turn predict fracture risk. Melhus et al. recently studied the frequency of VDR gene alleles in a small number (around 70 in each group) of osteoporotic vs non-osteoporotic Northern European women (36). While they found that a large number of the osteoporotic women were homozygous for the allele associated with low bone density (2.2-fold higher prevalence), this did not reach statistical significance. However, the interpretation of these results is limited by the relatively small sample size (37). Assuming a vertebral fracture prevalence of 15%, it can be estimated that a sample size of 230 subjects is required to have an 80% chance of detecting a statistical significance of the relative risk associated with the B allele observed by Melhus et al. (36). Therefore, the data from Melhus et al. do not preclude an effect of VDR gene alleles of osteoporosis but rather support the need for larger epidemiological studies in this area. Preliminary analyses of the relationship between bone density and genotype in the Dubbo Osteoporosis Epidemiology Study show a significant association between genotype and fracture incidence (White et al., manuscript submitted). Further full-scale studies in this and other prospective study groups will be of considerable interest.

Genetics disorders of bone collagen synthesis

While twin data support the genetic influence on the regulation of type I collagen turnover, there is a large body of data regarding specific type I collagen gene defects that result in osteoporosis of varying degrees. Osteogenesis imperfecta is a genetic disorder of bone with manifestations ranging from severe skeletal fragility and fetal death to milder forms expressing the phenotype of osteoporosis, blue sclerae and dentinogenesis imperfecta (38, 39). Because mutations in the collagen gene are known to result in bone fragility, it has been postulated that other collagen gene mutations may play a role in some forms of familial osteoporosis (38, 40). Because approximately 80% of the protein content of bone is type I collagen, it is to be expected that a defect in type I collagen would produce a clinical picture of fracture fragility. Indeed, collagen assembly follows a complex series of steps involving synthesis of procollagen, post-translational modification, assembly into the triple helical structure, propeptide cleavage and collagen self-assembly into fibrils. Studies in osteogenesis imperfecta have shown that even slight differences in amino acid sequence interfere with the triple helix formation, with severe effects on bone fragility. Spotila et al. assessed the two genes encoding type I procollagen (COL1A1 and COL1A2) in 26 subjects with low bone mass and a family history of osteoporosis, but without the full clinical criteria for the diagnosis of osteogenesis imperfecta (41). Three of the subjects had mutations that altered the encoded amino acid. Type I procollagen gene mutations may therefore explain some cases of low bone density and fracture and may also contribute to some extent to the population variability in fracture rates, although this has as yet not been examined. Whether variability in type I procollagen genes could contribute in part also to the genetic variability in bone turnover remains to be examined. Clearly, bone is a complex tissue and mutations in any of the many bone-specific structural proteins involved in mineralization and remodelling might be associated with osteoporosis.

Other genetic factors

A 49-kD glycoprotein, $\alpha_2$-HS glycoprotein (AHSG), is synthesized in the liver and has bone resorbing properties (42). There are many variants of AHSG that tend to be inherited in an autosomal co-dominant
fashion. Recent reports suggest a relationship between bone density in postmenopausal women and common AHSG phenotypes (42). However, there appear to be significant differences in oestradiol levels between the phenotypic groups that may contribute to this difference, and no significant relationship was found between the AHSG phenotype and bone turnover markers. Further work is required, perhaps in twins studies, to examine the role of the AHSG allelic variability and bone density in pre- and postmenopausal women, but these early data are encouraging.

Conclusions

Genetic mechanisms and factors in the determination of bone turnover and bone density have important implications for our understanding of the pathophysiology and treatment of osteoporosis. Identification of those individuals genetically “at risk” of osteoporosis opens up opportunities to influence life-time fracture risk at various stages throughout life, e.g. by maximizing peak bone mass in adolescence by dietary calcium supplementation or by targeting oestrogen replacement therapy more effectively at the menopause. Whether VDR gene allelic differences are associated with response to treatment for the various therapies used to treat osteoporosis also requires investigation. The functional significance of these VDR gene alleles in relation to the vitamin D endocrine system and bone metabolism also remains to be elucidated fully. In particular, further studies are necessary to identify the molecular and physiological mechanisms of the VDR gene effect in order to define optimal interventions to improve and maintain bone density and ultimately to reduce overall fracture incidence. The use of twins promises to continue to be a powerful tool to investigate osteoporosis and other genetically influenced quantitative traits related to “multi-factorial” diseases of ageing populations. This tool has allowed the identification of the contribution of common VDR gene alleles to the overall risk of low bone mass and osteoporotic fractures. This opens a new window onto the pathophysiological processes of low bone mass and offers opportunities for the development of more specific targeted prevention and treatment options.

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