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Genetic and environmental determinants of osteoporosis

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Abstract

Osteoporosis is a common, complex disease that is influenced by genetic and environmental factors. Although molecular genetic studies have identified several potential regions of linkage, underlying susceptibility gene(s) are largely unknown. Genetic susceptibility to osteoporosis may be both context dependent and developmentally regulated, and epigenetic mechanisms are the likely link between gene and environment. In this paper we will review the status of genetic research into osteoporosis, and present the evidence for gene-environment interaction in its pathogenesis. Finally, the current challenges and future directions of research will be briefly discussed.

Keywords: Genetics, Gene-by-environment Interactions, Osteoporosis

Epidemiology

Osteoporosis is a major public health issue worldwide that results in increased individual morbidity, mortality, need for hospital care, and dependency. The most common clinical outcomes are fracture of the spine, hip and wrist. Osteoporosis currently affects 200,000 female and 100,000 male Hong Kong Southern Chinese. The estimated life-time risk of a Chinese woman having an osteoporotic fracture is 32%[1]. It is projected that by 2050, more than 50% of the world's hip fractures will occur in Asia, mainly in China[2]. The risk factors for the development of osteoporosis and fractures involve genetic and environmental influences, as well as an interaction between the two. Figure 1 shows a common model of susceptibility to osteoporosis and fracture.

This review discusses the complex interplay of genetic and environmental factors that may contribute to osteoporosis. We first review the status of genetic research into osteoporosis, and then present the evidence for gene environment interaction in its pathogenesis. Finally, current challenges and future directions of research will be briefly discussed.

Genetic studies

Bone mineral density (BMD) has widely been used as the criterion for the diagnosis of osteoporosis and a surrogate

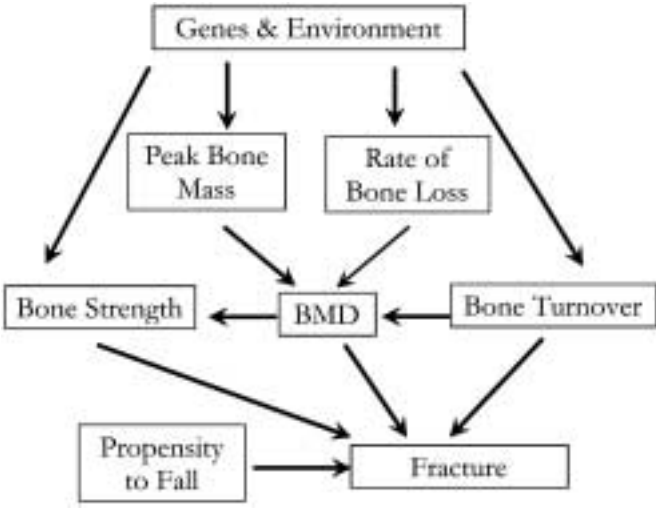

Figure 1. Common model of susceptibility to osteoporosis and fracture.

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VDR	LEPR	P57(KIP2)	IL-1RA
ERα	PLOD1	IGF-II	CASR
ERβ	QPCT	DRD4	CLCN7
COL1A1	CYP1B1	FRA-1	CT
TGFβ	LCT	TCIRG1	PTH
OPG	I-TRAF	MMP-1	BGP
LRP5	CCR2	MGP	ApoE
IL-6	PPARG	CYP1A1	CYP17
IGF-1	PDE4	IL-10	Klotho
MTHFR	RIL	SERT	BMP2
CYP19	HLA-A	GH1	NCOA3
CTR	NPY	MMP-9	OSCAR
TNFR2	PON1	COMT	RUNX2
TNF α	WRN	IRAK1	AHSG
COL1A2	GnRH		PTHR1
AR			SOST
			PDE4D
			DBP

Figure 2. Genes associated with BMD/osteoporosis.

phenotype for genetic studies. It is well established that BMD is under strong genetic control. The search for genes that influence susceptibility to osteoporosis remains one of the greatest challenges and the most active area of research in bone biology. The most important finding, following a decade of hunting for genes that are involved in osteoporosis, is that such genes probably really do exist despite the key role of environmental factors. Nonetheless it is unlikely that there are a few genes with a major influence on BMD (>10% of the genetic variance in BMD). The genetic component of osteoporosis is determined by an assembly of multiple genes with small individual effects, each gene most likely responsible for less than 5% of the genetic variance in the general population. Both linkage and association methods have been used to identify genetic susceptibility loci to osteoporosis. A comprehensive review of the genetic basis of osteoporosis susceptibility has been published recently[3].

Candidate-gene association studies

The main advantage of an association analysis approach is that it is more powerful than linkage analysis for identifying genes with smaller effects. In addition, it is easier and less expensive to recruit large numbers of unrelated affected individuals than large numbers of pedigrees. The first and foremost challenge when searching for an association of genes with complex disease is related to statistical errors: false positives (α or type I errors) result chiefly from an unknown population substructure and may result in differences in allele frequencies between cases and controls that are unrelated to their disease status. False-negatives (β or type II errors) result primarily from the limited power of studies. Statistical methods are now available that allow for the detection of and correction for any imbalances between cases and controls, and association methods that use family-based controls have been developed to avoid issues of population substructure.

Following the description of the vitamin-D receptor (VDR) polymorphisms 10 years ago, the annual number of published genetic association studies has grown rapidly. The most noticeable differences in the present associations compared with those reported earlier are that they more often include larger samples, consider multiple variants and haplotypes, and include gene-gene and gene-environment interactions. For example, the Genetic Markers of Osteoporosis (GENOMOS) project is a large-scale study of several candidate gene polymorphisms in relation to osteoporosis-related outcomes in individuals drawn from several European centers[4]. It evaluated the role of the gene encoding the alpha 1 chain of type 1 collagen (*COLIA1*) Sp1 alleles as a predictor of BMD and fracture, involved 20,786 individuals, and demonstrated that the *COLIA1* Sp1 polymorphism is associated with reduced BMD and could predispose to incident vertebral fractures in women, independent of BMD. By the end of 2005, 63 genes had been associated with BMD and osteoporosis-related phenotypes, 16 genes had been replicated in more than 4 independent samples, 18 genes in 1-3 independent samples, and 29 genes were associated with BMD in just a single sample (Figure 2).

Previous results from association studies of candidate genes based on one or a few markers have often been inconsistent and unreliable. Meta-analysis provides a means of resolving these discrepancies and has been successfully used to define the role of several candidate genes in osteoporosis[5-11]. Nonetheless with the availability of millions of single nucleotide polymorphisms (SNPs) in public databases, rapid improvements in SNP genotyping technology and the completion of the International Haplotype Map (HapMap) Project[12], research is moving towards systematic testing of large numbers of candidate genes or anonymous variants. A recent large-scale association study that investigated more than 25,000 SNPs located within 16,000 genes established that variants in the gene encoding the phosphodiesterase 4D account for some of the genetic contribution to BMD variation in humans[13]. In future, genome-wide association studies in large populations of cases and controls will be the standard approach to identify genes involved in complex diseases.

Family-based linkage studies

Replication of linkage regions

The main advantage of linkage studies has been their ability to identify both novel genes or pathways and genes with larger phenotypic effects. More than 10 genome-wide linkage scans in humans have been performed and have identified a few significant or suggestive linkage regions for BMD[14-28]. Some regions have been replicated in multiple studies. These include 1p36, 1q21-23, 2p23-24, 3p21-24,

Adverse risk factors	Protective factors
Smoking	High phytoestrogen intake
Alcoholism	Sports activity
Low calcium intake	Pregnancy
Vitamin D insufficiency	
Low body weight	
- BMI < 18-20 kg/m^2	
Estrogen deficiency	
- delayed menarche	
- early menopause	
- bilateral ovariectomy	
- premenopausal amenorrhea	
High parathyroid hormone	
History of fracture	
- personal and in first-degree relative	
Caucasian race	
Advanced age	
Female	
Dementia	

Table 1. Clinical risk factors for osteoporosis.

4q25, 4q32-34, 6p11, 7p14, 11q14-23, 12q23-24, 13q31-34, and 20p12[3]. A recent meta-analysis of genome-wide linkage scans in osteoporosis revealed that the region most strongly supported is chromosome 16p, although support was also obtained for regions on chromosomes 1p, 3p, 6, 10, 18, 20p, and 22q[29]. Identification of the underlying susceptibility gene(s) and sequence variations in these regions will remain a major challenge.

Positional cloning of BMP2

The first osteoporosis gene identified by linkage and positional cloning in humans was the bone morphogenetic protein 2 gene (*BMP2*), an important regulator of osteoblast differentiation. A significant linkage signal on 20p12 to osteoporosis risk was detected in an Icelandic population[24]. Subsequent linkage disequilibrium mapping indicated that the *BMP2* gene is significantly associated with osteoporosis risk as well as BMD and may account for part of the observed linkage signal. An independent association study in a group of Danish postmenopausal women confirmed this finding.

Environmental factors

Lifestyle and anthropometric factors

Lifestyle factors associated with low BMD and osteoporosis include smoking, alcohol intake, low calcium intake, vitamin D insufficiency, low phytoestrogen intake, estrogen deficiency, delayed menarche, low body weight, and physical inactivity (Table 1). A large study of 116,229 female nurses aged 34-59 years at baseline and followed for 12 years showed a trend towards an increased risk of hip fracture in current smokers. The relative risk was 1.3 compared with subjects who had never smoked. A benefit from stopping smoking was seen 10 years after cessation (relative risk 0.7; 95% CI 0.5-0.9)[30]. In men, current smoking increases bone resorption without increasing formation[31]. Past smokers have a lower BMD than those who have never smoked, but not increased resorption. Cigarette smoke extract inhibits *in vitro* differentiation of human osteoprogenitor cells to osteoblast-like cells[32]. In a study of 297 women who drank alcohol and 148 who did not, moderate alcohol consumption (>28.6 g/week) was significantly associated with a higher BMD at the lumbar spine and distal radius. Markers of bone turnover were also reduced in drinkers, as were levels of parathyroid hormone, suggesting that alcohol may reduce bone remodelling[33]. Nonetheless a large study of 17, 868 men and 13,917 women, that pooled data from three population studies in Copenhagen, Denmark, from 1964 to 1992, demonstrated that alcohol intake above the current recommended allowance (1-27 drinks per week for men and 1-13 for women) was associated with a dose-dependent increasing risk of hip fracture[34]. Women showed a trend towards higher risk with 14 to 27 drinks per week, and drinking beer appeared to increase the risk of hip fracture more than wine and spirits.

When dietary calcium and/or vitamin D levels are inadequate, calcium absorption is impaired and there is a compensatory increase in parathyroid hormone (PTH) levels. This results in increased bone resportion and accelerated bone loss[35]. It has been suggested that serum 25(OH)D levels >30 ng/mL are required for maximal calcium absorption and optimal health[36]. Supplementation with vitamin D and calcium significantly increases BMD, increases serum 25(OH)D, decreases serum parathyroid hormone and reduces fracture risk[37-38]. We have demonstrated that a high dietary phytoestrogen intake is associated with higher BMD and lower PTH levels in postmenopausal but not premenopausal women in 650 Chinese women aged 19 to 86 years[39].

We studied the effects of lifestyle on low BMD/osteoporosis in 418 southern Chinese women, aged 20-39 years, in an attempt to identify clinical predictors of bone mass in young premenopausal women[40]. Low body weight (<44 kg) was associated with an 8.3-fold and a 6.8-fold risk of having low aBMD at the spine and hip, respectively. A body height below 153 cm was associated with a 4.8-fold risk in the small L2-4 bone area and a 3.9-fold risk in the small femoral neck area. Delayed puberty (onset of menstruation beyond 14 years) was associated with a 2.2-fold increased risk of having low aBMD at the hip. Physical inactivity was associated with a 2.8-fold risk of low spine vBMD and a 3.3-fold risk of low hip aBMD. Pregnancy protected against low spine aBMD and spine vBMD, low femoral neck vBMD and small L2-4 bone area vBMD.

Gender as an environmental factor

The molecular, cellular and physiological milieus differ between male and female fetuses throughout life. Thus gender may be considered an environmental factor that can influence disease risk in a genotype-specific manner, and may be an important consideration in diseases with skewed sex ratios such as osteoporosis. In humans, it is well known that individual bone strength differs between men and women[41]. We estimated the heritability of BMD in 3,320 southern Chinese from 1,019 families using the variance components model and found significant gender difference in the genetic variance of BMD at the hip but not the spine[42]. The narrow heritability for age, weight and height-adjusted BMD was 0.63-0.71 for females, and 0.74-0.79 for males. In studies of recombinant strains of mice, nine quantitative trait loci (QTLs) for total body bone mass were identified in males and seven in females[43]. Only two of the QTLs were shared between the two sexes. Several studies have demonstrated that for the most part, different genes in men and women regulate bone mass[19,26,44-46]. We recently identified two gender-specific QTLs for hip BMD variation in chromosome 11q in Chinese. For women, a QTL that affects femoral neck BMD was found on 11q21 near the marker D11S4175. For men, a QTL that affects femoral neck BMD was located on 11q24 near the marker D11S4126[47].

Gene-environment interactions

Gene-environment interactions are probably involved in most complex diseases[48]. A specific genotype may result in a phenotype only in certain environments, and result in a different phenotype in a different environment. IL-6 genetic variation was prominently associated with hip BMD in late postmenopausal women, those without estrogen replacement therapy, and those with inadequate calcium intake[49]. The relationship between lumbar spine BMD and VDR genotype has been shown to vary with birth weight[50]. Among individuals in the lowest third for birth weight, spine BMD was higher in individuals with genotype 'BB' after adjustment for age, gender and weight at baseline. In contrast, spine BMD was reduced in individuals of the same genotype but in the highest third of birth weight distribution. A significantly statistical interaction was found between VDR genotype and birth weight as determinants of BMD. The relationship between weight in infancy and adult BMD has been replicated in population studies in the United States, Australia and Scandinavia[51]. These results suggest that genetic influences on BMD may be modified by poor nutrition *in utero*.

Developmental origins of osteoporosis

Evidence for developmental origins of osteoporosis is now accumulating. First, birth weight and weight in infancy were identified as predictors of basal levels of growth hormone and cortisol during late adult life[52-54]. Interactions between the genome and early environment might establish basal levels of circulating growth hormone and thereby contribute to accelerated bone loss. A significant interaction was observed between weight at 1 year, allelic variation at the human growth hormone gene, and bone loss rate[55]. Second, after adjustment for gender and gestational age, neonatal bone mass was strongly positively associated with birth weight, birth length, placental weight, maternal and paternal birth weights and maternal triceps skinfold thickness at 28 weeks. Maternal smoking and maternal energy intake at 18 weeks' gestation were negatively associated with neonatal bone mineral content for both the spine and the whole body[56]. Reduced maternal height, lower preconception maternal weight, reduced maternal fat stores during late pregnancy, history of maternal smoking, and lower maternal social class were all associated with reduced whole-body BMC of the children at age 9 years. Third, studies of 3,639 men and 3,447 women born in Helsinki University Central Hospital during 1924-1933 revealed that after adjustment for age and gender, there were two major determinants of hip fracture risk: tall maternal height and low rate of childhood growth[57]. Moreover, hip fracture risk was elevated among babies born short. Fracture subjects were shorter at birth but of average height by age 7 years. These results demonstrate that osteoporosis risk might be modified by environmental influences during early life.

Epigenetics: the mechanistic link between genes and the environment

Epigenetics refers to stable and heritable changes in gene expression that do not involve changes in DNA sequence. Many environmental factors interact with genes through epigenetic mechanisms, and these interactions act primarily during early life. In the agouti mouse mutant, maternal dietary folate supplementation at conception alters the expression of the imprinted agouti gene by altering the capacity for methylation[58]. The choice of exon usage in the glucocorticoid receptor gene is altered both by prenatal glucocorticoids and neonatal behavioural manipulation owing to changes in histone acetylation and DNA methylation in a transcriptional factor binding site[59]. Epigenetic mechanisms that respond to *in utero* or early life exposures play a critical role in influencing susceptibility to asthma and allergy[60]. Several environmental factors have been shown to directly influence methylation patterns and expression levels of some genes, and, in some cases, epigenetic alterations are transmissible beyond a single generation[61]. The role of epigenetics in the pathogenesis of osteoporosis remains to be elucidated.

Conclusions and future directions

We are gradually coming closer to unravelling many of the determinants of osteoporosis and fracture risk. There is evidence for a significant genetic contribution interacting with

environmental factors. Revealing the complex interactions that underlie osteoporosis susceptibility is challenging, and will require study of large samples of well-characterized subjects. One of the most intriguing findings is that environmental influences on the fetus appear to affect adult skeletal status. It is essential that present and future genetic studies include high-quality environmental data in the analytical process. Further work to elucidate the mechanisms of gene environment interaction, and thus potentially provide ways to reduce this devastating public health problem, is vital.

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