REVIEW

Molecular studies of identification of genes for osteoporosis: the 2002 update

Yao-Zhong Liu¹, Yong-Jun Liu¹, Robert R Recker
and Hong-Wen Deng^{1,2}

Osteoporosis Research Center, Creighton University, 601 N 30th St, Suite 6787, Omaha, Nebraska 68131, USA

¹Department of Biomedical Sciences, Creighton University, 601 N 30th St, Suite 6787, Omaha, Nebraska 68131, USA

²Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, ChangSha, Hunan 410081, Republic of China
(Requests for offprints should be addressed to H-W Deng; Email: deng@creighton.edu)

Abstract

We aim to give a comprehensive review, updated to 2002, of the most important and representative molecular genetic studies, performed mainly within the past decade, that aimed to identify the gene(s) involved in osteoporosis. Early reviews were largely confined to association studies in humans, but we review here, separately, the results of both association and linkage studies in humans, and quantitative trait locus (QTL) mapping in animal models. The main results of all the studies are tabulated for comparison and ease of reference, and to provide a comprehensive retrospective view of molecular genetics studies of osteoporosis. The most

striking findings and the most representative studies are singled out for comment regarding the immediacy of their influence on present understanding of the genetics of osteoporosis and on the current status of genetic research in osteoporosis. This is particularly relevant for studies on the association of the vitamin D receptor (*VDR*) gene, for which there has been a large body of studies and reviews published. The format adopted by this review should be ideal for accommodating future new advances and studies in a fairly young field that is still developing rapidly.

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Introduction

The genetics of osteoporosis represents one of the most active areas for research in bone biology. With many new publications emerging each year, the field of osteoporosis genetics research has a pressing need for periodical reviews lending an up-to-date and objective retrospective analysis and overview on both the methodologies for, and the discoveries made as a result of, the endeavours to identify the genetic factors and gene–environment interactions underlying osteoporosis. A few review articles are available that address the role of genetic factors in osteoporosis (Audi *et al.* 1999, Eisman 1999, Zmuda *et al.* 1999a, Stewart & Ralston 2000, Rizzoli *et al.* 2001, Peacock *et al.* 2002b, Ralston 2002) and strategies for the search for osteoporosis genes (Rogers *et al.* 1997, Nguyen & Eisman 2000, Nguyen *et al.* 2000, Blank 2001). Recently, Recker & Deng (2002) reviewed the current methodologies of gene mapping for osteoporosis on the basis of their experience with molecular genetics in relation to bone, and the use of statistical genetics in the identification of genes for complex traits. As a complement to that, the present review offers a comprehensive overview of the important findings

made within the past decade in the field of genetics of osteoporosis. The results of important studies are entered in Tables for the purpose of comparison and ease of reference. Table 1 summarizes the major candidate genes subject to association studies. Tables 2 and 3 include association studies that produced positive and negative results respectively. Table 4 summarizes studies on risk prediction with genetic factors for osteoporosis and other related diseases. Table 5 highlights the studies on gene-by-gene or gene-by-non-genetic factor interactions. Given that, currently, there is no detailed review dedicated to studies using the approaches of linkage analysis in humans and quantitative trait locus (QTL) mapping in animal models in osteoporosis research, these studies are elaborated upon and summarized individually in this article in Tables 6, 7, 9 and 10 respectively. The issue regarding the statistical power of the current linkage analysis is summarized in Table 8 and Fig. 1.

Comments are made on the most representative findings for their immediate influence on the understanding of the genetic basis of osteoporosis and on current trends in genetic research in osteoporosis. This is particularly relevant for association studies of the vitamin D receptor

Table 1 Major candidate genes showing association to bone mass variation

	Candidate gene	Protein	Chromosome location	Reference
Biological classification Calcitropic hormones and receptors	<i>VDR</i>	Vitamin D receptor	12q12-14	Morrison <i>et al.</i> 1994
	<i>ER-α</i>	Estrogen receptor-α	6q25	Sano <i>et al.</i> 1995
	<i>ER-β</i>	Estrogen receptor-β	14q22-24	Ogawa <i>et al.</i> 2000
	<i>CT</i>	Calcitonin	11p15	Miyao <i>et al.</i> 2000a
	<i>CTR</i>	Calcitonin receptor	7q21	Masi <i>et al.</i> 1998
	<i>PTH</i>	Parathyroid hormone	11p15	Hosoi <i>et al.</i> 1999
	<i>PTHrP</i>	Parathyroid hormone receptor 1	3p22-21	Minagawa <i>et al.</i> 2002
	<i>CYP19</i>	Aromatase	15q21	Masi <i>et al.</i> 2001
	<i>GCCR</i>	Glucocorticoid receptor	5q31	Huizenga <i>et al.</i> 1998
	<i>CaSR</i>	Calcium-sensing receptor	3q13-21	Tsukamoto <i>et al.</i> 2000a
Cytokines, growth factors and receptors	<i>AR</i>	Androgen receptor	Xq11-12	Sowers <i>et al.</i> 1999
	<i>TGF-β1</i>	Transforming growth factor-β1	19q13	Langdahl <i>et al.</i> 1997
	<i>IL-6</i>	Interleukin-6	7p21	Murray <i>et al.</i> 1997
	<i>IGF-I</i>	Insulin-like growth factor I	12q22-24	Miyao <i>et al.</i> 1998
	<i>IL-1ra</i>	Interleukin-1 receptor antagonist	2q14	Keen <i>et al.</i> 1998
	<i>OPG</i>	Osteoprotegerin	8q24	Arko <i>et al.</i> 2002
	<i>TNF-α</i>	Tumor necrosis factor-α	6p21	Fontova <i>et al.</i> 2002
Bone matrix proteins	<i>TNFR2</i>	Tumor necrosis factor receptor 2	1p36	Spotila <i>et al.</i> 2000
	<i>COL1A1</i>	Collagen type I α1	17q21-22	Grant <i>et al.</i> 1996
	<i>COL1A2</i>	Collagen type I α2	7q22	Suuriniemi <i>et al.</i> 2002
	<i>BGP</i>	Osteocalcin	1q25-31	Dohi <i>et al.</i> 1998
	<i>MGP</i>	Matrix Gla protein	12p13-12	Tsukamoto <i>et al.</i> 2000b
Miscellaneous	<i>AHSG</i>	α-2-HS-glycoprotein	3q27	Dickson <i>et al.</i> 1994
	<i>ApoE</i>	Apolipoprotein E	19q13	Shiraki <i>et al.</i> 1997
	<i>MTHFR</i>	Methylenetetrahydrofolate reductase	1p36	Miyao <i>et al.</i> 2000b
	<i>P57(KIP2)</i>	Cyclin-dependent kinase inhibitor 1c	11p15	Urano <i>et al.</i> 2000
	<i>HLA-A</i>	Major histocompatibility complex, class I, A	6p21	Tsuji <i>et al.</i> 1998
	<i>PPAR-γ</i>	Peroxisome proliferator-activated receptor-γ	3p25	Ogawa <i>et al.</i> 1999
	<i>FRA-1</i>	Fos-related antigen-1	11q13	Albagha <i>et al.</i> 2002
	<i>RUNX-2</i>	Runt-related transcription factor-2	6p21	Vaughan <i>et al.</i> 2002
	<i>Klotho</i> gene	Klotho protein	13q12	Kawano <i>et al.</i> 2002
	<i>WRN</i> (Werner syndrome gene)	Werner helicase	8p12-11	Ogata <i>et al.</i> 2001

(*VDR*) gene, on which numerous studies have been published and several other in-depth review articles are available (Ferrari *et al.* 1998c, Eisman 1999, Stewart & Ralston 2000, Rizzoli *et al.* 2001, Peacock *et al.* 2002b, Ralston 2002).

Association studies

The genetic study of osteoporosis has been based largely on research into candidate genes relevant to bone metabolism (Table 1). The *VDR* gene, the collagen type I α1 (*COL1A1*) gene, and the estrogen receptor-α (*ER-α*) gene are among those most intensively studied.

VDR gene

Polymorphisms at four marker loci within the *VDR* gene have been identified as related to biological variations in

bone mass. All these polymorphisms are restriction fragment length polymorphisms (RFLPs). Recently, a novel polymorphism in the binding site for Cdx-2 (an intestine-specific homeodomain-containing transcription factor) in the *VDR* gene promoter region was also associated with variation in bone mineral density (BMD) in a Japanese population (Arai *et al.* 2001).

The association of the *VDR* gene polymorphism with bone turnover was first revealed in a study of 91 individuals of white British-Australian origin (Morrison *et al.* 1992). In that study, the *VDR* gene polymorphism was predictive of circulating osteocalcin concentrations, such that the concentrations of osteocalcin in BB individuals of the BsmI polymorphism were significantly greater than those in bb individuals. Similar effects were also found for the ApaI and the EcoRV RFLPs. The result was complemented by a subsequent study (Morrison *et al.* 1994) that identified the relationship of the BsmI polymorphism of the *VDR* gene to bone density variation – namely, that

individuals with the bb genotype had significantly greater bone density than others. Differences in bone density predicted by this association accounted for up to 75% of the total genetic effect on bone density in normal individuals. Although these findings were overestimated as a result of genotyping errors (Morrison *et al.* 1997), they stimulated a host of studies on the effects of *VDR* gene polymorphisms on bone-related traits in a variety of populations (Table 2) and sparked a new era of research into the identification of genes for osteoporosis.

After the study by Morrison *et al.* (1992), several association studies in white populations confirmed the influence of the *VDR* gene polymorphism on variation in BMD (Table 2). In a population of 470 premenopausal women, spinal and trochanter BMD were significantly correlated with BsmI polymorphism (Salamone *et al.* 1996). Association with BMD was also detected for polymorphisms at other marker loci of the *VDR* gene, such as the TaqI (Spector *et al.* 1995) and FokI (Gross *et al.* 1996) polymorphisms. The association even held good for different races, as demonstrated by a study in 202 premenopausal Japanese women (Tokita *et al.* 1996).

A series of studies aimed to unravel the underlying mechanisms of the effects of the *VDR* gene on bone metabolism that lead to the eventual variation in bone phenotype. Association studies in which relevant bone biochemical markers were used as surrogate phenotypes have illustrated the *VDR* gene polymorphism in various aspects of bone metabolism. Studies of the concentrations of markers of bone turnover revealed that the state of bone turnover differed across *VDR* genotypes (Morrison *et al.* 1992, Tokita *et al.* 1996, Lorentzon *et al.* 2001, Sheehan *et al.* 2001). In the study by Tokita *et al.* (1996), the Bb genotype was associated with a state of both greater bone turnover and lower BMD values than its counterpart bb, supporting the concept that the regulatory effect of the *VDR* gene on bone and mineral metabolism leads to variation in bone mass as the end-point phenotype.

The influence of *VDR* gene polymorphism on Ca^{2+} homeostasis has also been extensively studied. In 72 healthy children, the FokI polymorphism was related to calcium absorption (Ames *et al.* 1999). The FF individuals had both greater mean calcium absorption and greater BMD values than did Ff and ff individuals. This may suggest a differential action of the *VDR* alleles on intestinal calcium absorption, which in turn leads to the different degrees of accrual of BMD in these children. Such variation in calcium absorption was further defined in a study of 60 postmenopausal women (Dawson-Hughes *et al.* 1995). When calcium intake was limited, a lower fractional calcium absorption occurred in BB individuals compared with bb individuals. This inefficient calcium absorption in the BB genotype is a reflection of functional variation in the intestine *VDR* that may be determined by the *VDR* gene polymorphism. The modulation of intestinal calcium absorption by *VDR* polymorphism was

confirmed in a group of postmenopausal Thai women (Ongphiphadhanakul *et al.* 1997) and in two independent studies on the ApaI and TaqI polymorphisms (Gennari *et al.* 1997, Wishart *et al.* 1997).

A more comprehensive view of the influence of the *VDR* gene on bone homeostasis was achieved in a study of 21 premenopausal women (Howard *et al.* 1995). The BB genotype, in comparison with the bb genotype, had a greater set point for feedback inhibition of parathyroid hormone (PTH) initiated by an increase in 1,25-dihydroxyvitamin D ($1,25\text{-(OH)}_2\text{D}$), more active bone resorption and breakdown of type I collagen, and greater concentrations of $1,25\text{-(OH)}_2\text{D}$ required to maintain normocalcemia. These findings indicate possible differences between *VDR* genotypes with respect to the quality or quantity of VDRs in different target tissues (Dawson-Hughes *et al.* 1995, Ongphiphadhanakul *et al.* 1997).

A dynamic picture of bone growth was provided by studies on variations, not only in childhood bone accrual and adolescent peak BMD, but also in age-related bone loss. In a study on prepubertal girls, the AA genotype of the ApaI polymorphism and the BB genotype of the BsmI polymorphism were associated with lower BMDs than were found in the aa and bb individuals (Sainz *et al.* 1997). Such a difference in BMD between the BsmI genotypes might persist through adolescence, given the findings of another study of 75 young Finns, among whom those with the genotype bb had significantly greater peak bone mass than did those with the BB genotype (Viitonen *et al.* 1996). Peak bone mass was also found to be correlated with the ApaI, BsmI and TaqI polymorphisms in 677 white women, although in this case the BB rather than the bb individuals had the greatest peak bone mass (Rubin *et al.* 1999). Similarly, age-related bone loss differs between the *VDR* BsmI genotypes. In a group of elderly individuals, Ferrari *et al.* (1995) found that those with the BB genotype lost bone, whereas no significant bone loss was suffered by the bb individuals. Such a trend of association was also detected in a population sample of larger size (Brown *et al.* 2001), in whom the individuals TT for the TaqI polymorphism had lower rate of bone loss than those with other genotypes. Given the tight linkage disequilibrium of the b allele with the T allele, the bb individuals might also be those with less bone loss. The TT and bb genotypes were associated with less bone loss in two other independent studies, one in patients with early rheumatoid arthritis and another in postmenopausal Japanese women (Gough *et al.* 1998, Kikuchi *et al.* 1999).

A tentative conclusion that can be made on the basis of the data summarized so far in this text is that the bb or TT genotypes of the *VDR* gene are more advantageous in terms of bone metabolism, calcium homeostasis, bone accrual during childhood and bone 'retention' later in life. Conversely, BB or tt individuals might be those more likely to develop osteoporosis. With progressively expanding new data, such a contention may not always hold, as

Table 2 Association studies with positive findings

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
VDR	Bsml	250	Normal healthy white twins	BMD of LS, FN, Ward's triangle (bb>Bb>BB)	LS (0.000054), FN (0.038), Ward's triangle (0.033)	Morrison et al. 1994
	Bsml, Apal, EcoRV	91	White individuals	Osteocalcin concentrations (BB>bb; AA>aa; EE>ee)	BB vs bb: 0.0001 AA vs aa: 0.001 EE vs ee: 0.015	Morrison et al. 1992
	TaqI	190	95 dizygotic twins, aged 50–69 yr postmenopausal	BMD (lumbar, FN, Ward's triangle, total body) TT>tt	Total body: 0.007 Ward's: 0.008 Lumbar: 0.033 Femoral: 0.034	Spector et al. 1995
	Bsml, Apal, TaqI	202	Normal healthy premenopausal Japanese women	BMD (Bb<bb; BbAA>Tt<bbaaTT) Bone turnover: levels of osteocalcin, alkaline phosphatase, 1,25-dihydroxyvitamin D (Bb>bb)		Tokita et al. 1996
	Bsml, Apal, TaqI	1782	Men and women aged 55–80 yr	FN BMD (bAT<other haplotypes)	0.04	Uitterlinden et al. 1996
	Bsml	470	Healthy premenopausal women, aged 44–50 yr	Spinal and trochanter BMD (BB>Bb, bb)		Salamone et al. 1996
	Bsml	171	Pre- and postmenopausal women	FN bone density (bb<BB)	<0.02	Houston et al. 1996
	Apal, Bsml, TaqI	100	Normal prepubertal American girls of Mexican descent	Femoral BMD (aa>AA; bb>BB) Lumbar vertebral BMD (aa>AA; bb>BB) Whole-group FN BMD (ff<FF) White: total body BMD, FN BMD (ff<FF)	0.008 (for aa>AA) 0.04 (for bb>BB) 0.01 (for aa>AA) 0.03 (for bb>BB) 0.015 0.042 (total body BMD) 0.001 (FN)	Sainz et al. 1997
	FokI	154	Premenopausal American women (72 black and 82 white) 20–40 yr	LS BMD (ff<FF) Decrease in BMD at FN in 2 yr (ff>FF)	0.001 0.005 <0.05	Harris et al. 1997
	FokI	100	Postmenopausal Mexican-American white women	LS BMD (mm>MM)		Gross et al. 1996
	FokI	110	Premenopausal Japanese women	FN BMD (BB lowest among women >10 yr menopausal), rate of bone loss over 2 yr at spine, FN, radius (BB>other)		Arai et al. 1997
	Bsml	229	Healthy postmenopausal women	LS BMD loss over 18 months (BB, loss; bb, no loss; Bb, change correlated with calcium intake) Change in LS BMD in reaction to calcium intake (Bb)	0.01	Krall et al. 1995
	Bsml	72	Elderly individuals	Fractional calcium absorption at low calcium intake (BB<bb) Effect of calcium intake on BMD increase only in bb subjects FN BMD (bb>BB)	<0.05 for BB, <0.03 for Bb <0.03 (for Bb) 0.044 <0.05	Ferrari et al. 1995 Dawson-Hughes et al. 1995 Kiel et al. 1997

Continued

Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
VDR	Bsml	84	Thai postmenopausal women	24 h urinary calcium excretion (bb>Bb)	0.05	Onghiphadhanakul et al. 1997
	FokI	72	Healthy children aged 7–12 yr	BMD (FF>Ff>ff)	0.02	Ames et al. 1999
	Bsml	32	Healthy postmenopausal women	Calcium absorption (FF>Ff>ff)	0.04	Barger-Lux et al. 1995
	Apal, Bsml, Taql	558	Non-obese postmenopausal women (BMI <30 kg/m ²)	BMC (bone mineral content), weight (bb>Bb>BB)		Vandevyver et al. 1997
	Bsml	75	Young Finns, 20–29 yr	FN BMD (bb>BB)	0.04	
				Peak bone mass	LS, 0.030	Viitinen et al. 1996
	Bsml	83 (white)		BMD in LS, FN (bb>BB)	FN, 0.049	
		72 (black)		FN BMD (whole group) (bb, Bb>BB)	FN BMD (0.034)	Fleet et al. 1995
	Apal, Bsml, Taql	426	Italian postmenopausal women	Lumbar BMD (AAB8tt<aabbTT)	LS BMD 0.036)	Gennari et al. 1998
	Bsml	118	Old people (60–90 yr) with calcium intakes >800 mg/day	Trochanter BMD (bb>Bb, BB)	0.01	Kiel et al. 1997
		94	18–68 yr	FN BMD (bb>BB)	<0.05	
		50	Men from the above population	FN BMD (bb>BB)	<0.05	
	Bsml	380	Healthy women older than 70 yr, non-obese (BMI <30 kg/m ²)	FN BMD (bb>BB)	<0.05	Geusens et al. 1997
			Brazilian women, 20–47 yr, premenopausal	Quadriceps strength (bb>BB)	<0.01	
	Bsml	127	Japanese women, healthy, 43 ± 17 yr	Grip strength (bb>BB)	<0.05	Lazaretti-Castro et al. 1997
	Bsml	92	Japanese women, osteoporosis, 71 ± 10 yr	LS BMD (bb>BB), FN BMD (bb>Bb>BB)	<0.05	Tamai et al. 1997
	Bsml	90	Japanese women, osteoporosis, 71 ± 10 yr	LS BMD (bb>Bb)	<0.035	Tamai et al. 1997
	Bsml	78	Brazilian patients with IDDM	LS BMD (BB>bb)	<0.025	Tamai et al. 1997
	Bsml	81	Old women, >70 yr	LS, FN BMD (BB<Bb, bb)		Hauache et al. 1998
	Bsml	21	Premenopausal	Increase of BMD in response to vitamin D supplementation (BB, Bb>bb)	0.03	Graafmans et al. 1997
VDR	Apal, Bsml, Taql	99	Premenopausal	Increase in serum osteocalcin after 7-day oral 1,25(OH) ₂ stimulation (BB<bb)	0.01	Howard et al. 1995
	Apal, Bsml, Taql	62	Primary hyperthyroidism (56 women and 6 men)	Radiocalcium absorption higher in bbaaTT and aa	<0.05	Wishart et al. 1997
	Apal, Bsml, Taql	62	Japanese patients with end-stage renal diseases	Ca ²⁺ -mediated PTH inhibition (baT>BAT)	<0.05	Carling et al. 1997c
	Bsml	589	Healthy infants	Intact-PTH (i-PTH) (aa>AA, Aa)	≤0.04 (i-PTH)	Yokoyama et al. 1998
	TaqI	66	Women with mean age 65.5 yr	Intact-osteocalcin (i-OC) (aa>AA, Aa)	≤0.03 (i-OC)	Suarez et al. 1997
	Bsml	146	Normal men aged 20–83 yr	Body length, weight, surface area (BB girls>bb girls, BB boys<bb boys)		
	Apal	120	Young girls aged 18–19 yr	Weight at the age of 1 yr (tt>Tt>TT)	0.04	Keen et al. 1997a
				Bone density in the forearm (BB<Bb, bb)	0.030 (bone density)	Need et al. 1996
				Bone area in the forearm (BB>Bb, bb)	0.026 (bone area)	
				BMD at distal radius	<0.05	Kitagawa et al. 1998
				Age at menarche (Aa<aa)		

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Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
VDR	Apal	171	Bangladeshi Asians at risk for type 2 diabetes	Insulin secretion index (AA>Aa>aa)	0.006	Hitman et al. 1998
	TaqI	232	Early rheumatoid arthritis patients	Bone loss at both LS and FN (tt>TT)	<0.05 (LS) <0.01 (FN)	Gough et al. 1998
	Bsml	170	Middle-aged white women	Femoral shaft expansion, increase in cortical area (bb>other)		Heaney et al. 1997
	Bsml	66	Japanese patients with primary hyperparathyroidism	BMD in radius (bb<BB, Bb)		Kobayashi et al. 1998
	Bsml	88	Japanese hemodialysed patients	Decrease of whole-body and FN BMD during 18 months (BB>Bb, bb)	<0.02	Karkoszka et al. 1998
	Apal, Bsml, TaqI	42	Patients with sporadic pHPT (primary hyperparathyroidism)	VDR and PTH mRNA levels (bb, aa, TT<BB, AA, tt for VDR; bb, aa, TT>BB, AA, tt for PTH mRNA)	<0.0001–0.02	Carling T 1998a
	Apal, Bsml, TaqI	120	White postmenopausal women aged 61 ± 0.6 yr	Intestinal Ca ²⁺ absorption (Bb, tt<bb, TT; AABbtt<aabbTT, AaBbTt)	0.0015	Gennari et al. 1997
	FokI	98	Postmenopausal osteoporotic French women aged 45–75 yr	FN BMD (ff<FF, Ff)	Significant	Lucotte et al. 1999
	Bsml	191	Postmenopausal Japanese women	BMD loss in both early and late postmenopausal women (Bb>bb)	0.001	Kikuchi et al. 1999
	Bsml	197	Prepubertal girls and peri- and postpubertal adolescents of white origin	LS BMD, height (BB<Bb, bb)	≤0.02	Ferrari et al. 1998a
	TaqI	68	Healthy girls aged 7 yr, white	LS BMD (TT>Tt>tt) Body weight and height (TT>tt)	0.038 (BMD) 0.03 (body weight) 0.008 (height)	Tao et al. 1998
	Bsml	90	Healthy white males	Height at birth (BB<Bb, bb), growth from birth to age 16.9 ± 0.3 (BB<Bb, bb) height during (age 16.9 ± 0.3) and after puberty (age 19.3 ± 0.7) (BB<Bb, bb), bone area of humerus, femur and total body (BB<Bb, bb)	0.01 (height at birth) 0.01 (growth after birth) 0.005–0.008 (height during and after puberty)	Lorentzon et al. 2000
	Apal, Bsml, TaqI	677	Healthy white women aged 18–35 yr	Peak BMD at femoral neck (B: 0<1<2; A: 0<1<2; T: 0>1>2)	<0.05 (bone area) 0.006 (Bsml), 0.005 (Apal), 0.001 (TaqI)	Rubin et al. 1999
	Bsml	104	Healthy young men, aged 24.3 ± 3.1 yr	BMD Z scores at LS and femoral trochanter (BB<Bb<bb)	0.001 (TaqI) 0.03 (LS), 0.05 (femoral trochanter)	Ferrari et al. 1999
	Apal, Bsml	99	Healthy white girls, aged 16.9 ± 1.2 yr	Circulating osteocalcin (Aa<aa), PTH (Aa>aa), LS BMD (Bb>bb, Tt>TT)	<0.01 (osteocalcin), 0.04 (PTH), 0.02 (BMD, for Bb vs Bb), 0.05 (BMD, for Tt vs TT)	Lorentzon et al. 2001
	TaqI	210	45 dizygotic twin pairs and 29 nuclear families containing 120 individuals	Rate of bone loss at FN and LS (bb or TT<other)	0.03	Brown et al. 2001
	TaqI, FokI	118	Healthy Irish adults aged 19–67 yr	Serum osteocalcin (tt>Tt>TT, ff>Ff>FF), urinary pyridinoline and deoxypyridinoline (ff>Ff>FF)		Sheehan et al. 2001
	Bsml	102	Late postmenopausal women aged 47–77 yr	LS BMD (bb>BB)		Marc et al. 2000

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Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
VDR	Bsml, Taql	399	192 osteoporotic patients, 207 normal controls	BMD of intertrochanteric region (bb>other, TT>other) and total hip (bb>other)	<0.001 (Bsml, intertrochanter), <0.01 (Bsml, total hip), <0.01 (Taql, intertrochanter)	Langdahl et al. 2000a
	FokI	400	Postmenopausal women of Italian descent, 164 osteoporotic, 117 osteopenia, 119 normal	Lumbar BMD	0.06	Gennari et al. 1999
	Bsml Bsml	326 24	Individuals of both sexes Late postmenopausal women	Lumbar and FN BMD of women (bb>Bb, BB) Response to cyclic etidronate therapy with calcium supplementation: LS BMD increase (BB, Bb>bb), osteocalcin decrease (bb>BB) Change in BMD in response to HRT (TT>Tt) Serum decrease in telopeptide of type I collagen (TT) LS (L2-4) BMD (GG<AA)		Gomez et al. 1999 Marc et al. 1999
	Taql	82	Japanese women aged 40-64 yr taking HRT for >1 yr		0.019 0.001	Kurabayashi et al. 1999
COL1A1	Cdx-2	261	Japanese women, aged 19-71 yr	LS (L2-4) BMD (GG<AA)	<0.05	Arai et al. 2001
	FokI	163	Postmenopausal Chinese women in Taiwan	LS BMD (Ff>ff)	0.029	Chen et al. 2002
	Bsml	171	Postmenopausal Chinese women in Taiwan	Lumbar and FN BMD	<0.001	Chen et al. 2001a
	M polymorphism, translation initiation site, exon 2	189	Pre- and perimenopausal Japanese women	Lumbar BMD (mm>Mm>MM)	<0.001	Kubota et al. 2002
	Bsml, Taql	303	Postmenopausal Korean women who received HRT for 1 yr	LS BMD annual percentage change (bb, TT<Bb, Tt)		Kim et al. 2002a
	Bsml	108	US mid-western postmenopausal white women	~ 5.8% spine BMD variation	0.004	Deng et al. 1999
	Sp1	299	White women, most postmenopausal, most osteopenic and osteoporotic	BMD (SS>Ss, ss)		Grant et al. 1996
	Sp1	1778	Postmenopausal	FN BMD, LS BMD (SS>Ss>ss) differences increase with age	<0.05	Uitterlinden et al. 1998
	Sp1	220	Healthy premenopausal women, 31-57 yr	Height (SS>ss), spine BMD, total body BMD, total body bone mineral content (BMC) (SS>Ss, ss), serum C-terminal extension propeptide of type I collagen (SS>Ss>ss)	0.05 (spine BMD), 0.046 (total body BMD), 0.02 (total body BMC) (disappear after adjustment with height), 0.03 (height), 0.04 (serum C-terminal extension propeptide of type I collagen)	Garnero et al. 1998

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Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
COL1A1	Sp1	185	White women, age 54.3 ± 4.6 yr	Reduction of LS BMD, increased risk of total fracture, increased urinary pyridinoline levels (association with carriage of 's' allele)	0.02 (LS BMD) 0.04 (risk of fracture) <0.05 (pyridinoline)	Keen et al. 1999
	Sp1	336	153 patients with hip fracture and 183 normal individuals	Femoral neck-shaft angle (Ss/ss>SS)	0.001	Qureshi et al. 2001
	Sp1	108	Perimenopausal women with osteopenia	Increase of FN BMD in response to bisphosphonate therapy (SS>Ss/ss)	0.002	Qureshi et al. 2002
	Sp1	352	Early postmenopausal Scottish women without HRT	Rate of bone loss over a 5–7 yr period at lumbar and FN (ss>SS/Ss)	0.004 (lumbar); 0.06 (FN)	MacDonald et al. 2001
	Sp1	185	Postmenopausal women	Fractures (more common in patients carrying 's' allele)	0.001	McGuigan et al. 2001
	Sp1	154	Postmenopausal Greek women	LS BMD (SS>Ss>ss)	0.056	Efstathiadou et al. 2001a
	Sp1	243	Individuals aged 65 yr and older	Total body loss in 5 yr (ss>Ss>SS)	0.009	Harris et al. 2000
	Sp1-RsaI haplotype	93 + 88	93 patients with vertebral fractures and 88 age-matched controls	Vertebral fracture	<0.05–0.001	McGuigan et al. 2000
	Sp1	715	663 postmenopausal (48–85 yr) and 52 perimenopausal (47–53 yr) Italian women	Age-adjusted hip BMD (SS>ss)	<0.05	Braga et al. 2000
	Sp1	109	Healthy prepubertal girls	BMD at the vertebral bones (SS>Ss>ss)		Sainz et al. 1999
ER-α	Sp1	210	45 dizygotic twin pairs and 29 nuclear families with 120 individuals	Rate of LS bone loss	0.0006	Brown et al. 2001
	Sp1	273	Community-dwelling healthy men (aged 71–86 yr)	Lower BMD at the forearm (for 's' allele)	0.03	Van-Pottelbergh et al. 2001a
	– 1997G/T in the promoter region	256	Postmenopausal women	Lower grip and biceps strength (for 's' allele)	0.03–0.04	
	Dinucleotide repeat polymorphism	146	Healthy postmenopausal Japanese women	Lumbar BMD	0.015	Garcia-Giralt et al. 2002
		144		FN BMD	0.044	Sano et al. 1995
				Spine BMD, total body BMD (allele C<other genotypes); serum intact osteocalcin, urinary pyridinoline, urinary deoxypyridinoline (allele C>other genotypes)	<0.01	
	XbaI	43	Premenopausal Japanese women	BMD (XX, Xx>xx)	<0.01	Mizunuma et al. 1997
	XbaI	30	Late premenopausal Japanese women	Serum N-region osteocalcin (XX, Xx>xx)	<0.05	Mizunuma et al. 1997
	PvuII, XbaI	238	Postmenopausal Japanese women aged 45–91 yr	BMD (PPxx<other, Px haplotype <other)	0.022 (LS BMD of PPxx) 0.002 (total body BMD of PPxx)	Kobayashi et al. 1996
				0.029 (LS BMD of Px haplotype)		

Continued

Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
<i>ER-α</i>	PvuII, XbaI	101	Postmenopausal patients with primary hyperparathyroidism	Serum calcium (PP<Pp, pp; XX<Xx, xx) LS BMD (PP<Pp, pp)	<0.01 (serum calcium of P) <0.05 (serum calcium of X) <0.05 (BMD)	Carling et al. 1997b
	TA repeat	261	Pre- and perimenopausal women, aged 28–48 yr	Total body calcium	<0.05	Sowers et al. 1999
	PvuII	54	Premenopausal Thai women	BMD at spine, FN, femoral trochanter, Ward's triangle (pp<Pp<PP)	<0.05	Ongphiphadhanakul et al. 1998b
	PvuII	900	Postmenopausal women, aged 55–80 yr	Onset of menopause (PP is 1.1 yr earlier than pp)	<0.02	Weel et al. 1999
	PvuII	253	Pre- and perimenopausal women	LS and total body BMD (pp>Pp>PP)	<0.005	Willing et al. 1998
	XbaI	248	Early postmenopausal women	LS (XX>Xx>xx)	<0.05	Salmen et al. 2000
	PvuII	177	without HRT	Decrease in BMD at LS (PP>Pp>pp)	0.002	
	PvuII	108	Healthy postmenopausal white women, aged 65 yr and over	BMI (PP>Pp>pp)	0.04	Deng & Chen 2000b
	XbaI PvuII	108	US mid-western postmenopausal white women	~ 3.5% FN BMD variation (for XbaI and PvuII) ~ 2.4% tbBMC variation	0.02 (FN BMD) 0.03 (tbBMC)	Deng et al. 1999
	PvuII, XbaI	229	Postmenopausal Korean women	LS BMD (pp<PP); FN BMD (Xx<xx)		Kim et al. 2001
	PvuII, XbaI	286	Postmenopausal Japanese women	Z score (PPxx<other), significant decrease of Z score over time (pp), significant increase of BMD in response to HRT (pp)	<0.05	Kobayashi et al. 2002
	PvuII, XbaI	300	Postmenopausal Japanese women aged 42–69 yr	LS BMD (PPXx<PPxx, ppxx)	<0.01 (PPxx), <0.05 (ppxx)	Ushiroyama et al. 2001
	PvuII, XbaI	206	Women from northeast Scotland	LS and FN BMD (carriers of Px haplotype<non-carriers)	0.002 (lumbar), 0.0006 (FN)	Albagha et al. 2001
	Codon 325	85	Late postmenopausal women	FN BMD (GG<CC)	0.029	Jurada et al. 2001
	TA repeat in the promoter region	378	160 women and 30 men with vertebral fractures, 124 women and 64 women as controls	LS and total hip BMD (low number of repeats<high number of repeats)	0.05	Langdahl et al. 2000b
	T262C polymorphism in exon 1	96	Postmenopausal Thai women	Increase in FN BMD in response to 2-yr estrogen treatment (only homozygotes)	<0.0001	Ongphiphadhanakul et al. 2001b
	PvuII	81	Thai men aged 20–79 yr	Lumbar BMD (P>p)	<0.05	Ongphiphadhanakul et al. 1998a
	PvuII	124	Thai postmenopausal women, 6–10 yr postmenopausal, with osteoporosis	BMD increase at L2–4 in response to estrogen therapy (pp<Pp, PP)	<0.05	Ongphiphadhanakul et al. 2000
	G2014A SNP in exon 8	106	Thai postmenopausal osteoporotic women aged more than 55 yr	Lumbar BMD	<0.001	Ongphiphadhanakul et al. 2001b
	(TA) _n dinucleotide repeat at the 5'	610	Postmenopausal women	Lumbar BMD [(TA<15)<other]	0.04	Becherini et al. 2000

Continued

Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
<i>ER-α</i>	TA dinucleotide repeat 5' upstream of exon 1	99	Postmenopausal Chinese women in Taiwan	FN BMD [(TA repeat ≥ 20) < (TA repeat ≤ 15)]		Chen et al. 2001b
	PvuII	91	10–12-yr old girls	24-month change of total body and total femur BMC (PP>Pp>pp)	0.049 (total body), 0.046 (total femur)	Suuniniemi et al. 2002
	XbaI	401 nuclear families	25–40-yr-old females	Spine BMD	0.054	Qin et al. 2003
	XbaI	5834	Women from 30 study groups (meta-analysis)	FN, LS, total body BMD (XX>other)		Ioannidis et al. 2002
<i>IL-6</i>	Minisatellite marker in the 3' flank of gene, six length variants from A to F	200	Pre- and postmenopausal women aged 45–88 yr	Bone mass at LS (F/F < C/F)	0.012	Murray et al. 1997
	CA repeat polymorphism	472	Postmenopausal Japanese women	BMD of radial bone (with A1 < without A1)	0.0221	Tsukamoto et al. 1999
	CA repeat polymorphism	79 complex pedigrees		Hip BMD	0.06	QY Huang et al. unpublished
	SNP at -634 174G→C	470 434	Japanese Healthy women aged 71.7 ± 5.7 yr	BMD (GG<GC<CC) CTx (bone resorption marker) (CC<GC<GG) BMD decrease with age (only in GG and GC)	0.05 0.006 <0.01	Ota et al. 2001 Ferrari et al. 2001
<i>IL-1ra</i>	IL-1 receptor antagonist gene (variable number tandem-repeat polymorphism)	108	Early postmenopausal women	Annual rates of change in spinal bone mass (associated with IL-1RN genotypes) Bone loss at spine (reduced for A2 allele carriers)	<0.05 (for association with IL-1RN genotypes) 0.05 (for association with carriage of A2 allele)	Keen et al. 1998
	86-base pair VNTR	389	Osteoporotic patients with vertebral fractures and normal controls	LS BMD (reduced in A1A1, A1A3)	0.014	Langdahl et al. 2000c
<i>TGF-β1</i>	VNTR	155	White Mediterranean women, 104 postmenopausal osteoporotic and 51 postmenopausal controls	Lumbar and hip BMD [A2 allele (A2+) > w/o A2 allele (A2-)]	0.02 (lumbar), 0.006 (hip)	Fontova et al. 2002
	T→C polymorphism in intron 5	1706	White female dizygotic twin aged 18–76 yr	FN BMD (A2A2<A1A1, A1A2)	<0.025	Keen et al. 2001
	713-8delC	161	Osteoporotic women	Increased bone alkaline phosphatase, increased excretion of hydroxyproline, reduced bone mass of LS	<0.05	Langdahl et al. 1997
	Signal sequence region T/C polymorphism	287	Postmenopausal Japanese women	LS BMD (CC>TT, TC), frequency of vertebral fracture (CC<TC, TT)	0.0001–0.027 (LS); 0.013 (frequency of vertebral fracture)	Yamada et al. 1998

Continued

Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
<i>TGF-β1</i>	Signal sequence region T29→C polymorphism	102	Estrogen-deficient postmenopausal women	LS and FN BMD (TT>CC)	0.05 (lumbar), 0.02 (FN)	Hinke et al. 2001
	C509→T	625	Postmenopausal Japanese women	Serum TGF-β1 (TT>CC)	0.001	Yamada et al. 2001
	T→C in exon 1	363	Postmenopausal Japanese women, divided into control group (n=130), vitamin D group (n=117), HRT group (n=116)	LS and total body BMD (TT<CT, CC)	TT>CC significant	Yamada et al. 2000
				LS bone loss rate (TT>TC>CC for control group)	Not significant	
<i>CTR</i>	713-8delC	256	Postmenopausal Italian women	Positive response to HRT of BMD (TT<TC<CC)		
				Positive response to vitamin D (only CC)		
				Hip BMD (Tt<TT, tt<Tt, Tt)	0.0001 (tt vs TT, Tt)	Bertoldo et al. 2000
				Fracture frequency (tt, Tt>TT)	0.006	
<i>CT</i>	Calcitonin receptor gene TaqI T→C mutation	201	Postmenopausal Italian women	Bone turnover		
				Lumbar BMD (tt<Tt)	0.005	Masi et al. 1998
				BMD at FN (Rt>rr, RR), fracture risk (Rr<rr, RR)		Taboulet et al. 1998
				Spine BMD (CC<others)	<0.05	Braga et al. 2000
<i>CT</i>	AluI restriction enzyme polymorphism	715	663 postmenopausal (48–85 yr) and 52 perimenopausal (47–52 yr) Italian women			
	CA repeat at the CT (calcitonin) locus	311	Japanese postmenopausal women (mean age 64.1 yr)	Lumbar BMD (10-CA-repeat allele<other)	0.04	Miyao et al. 2000a
	Androgen receptor (AGC) _n	261	Pre- and perimenopausal women, aged 28–48 yr	BMD of FN and LS	<0.001	Sowers et al. 1999
	Apolipoprotein E	284	Postmenopausal Japanese women aged 47–82 yr	LS BMD and total BMD (E4 -/->E4+/->E4+/+, serum intact osteocalcin (E4+/+>E4+/-, -/-)	0.031 (LS BMD); 0.022 (total BMD); 0.004 (osteocalcin)	Shiraki et al. 1997
<i>AHSG</i>	ApoE*4 allele	95	Healthy, peri- and postmenopausal white women	Annual spine bone loss (ApoE*4>no ApoE*4)	0.018	Salamone et al. 2000
	α ₂ -HS-Glycoprotein (AHSG)	88	Postmenopausal women	LS and FN BMD and levels of free estradiol (AHSG2>AHSG1)	<0.05	Dickson et al. 1994
				Calcaneal broadband ultrasound attenuation, height (AHSG2/2>1/2>1/1)	<0.05	Zmuda et al. 1998
				Z score for lumbar BMD and total body BMD (Bb<BB), bone turnover markers	0.014 (lumbar BMD), 0.040 (total body BMD), <0.05 (bone turnover)	Hosoi et al. 1999
<i>PTH</i>	PTH Bst B1	383	White women (age 66–92 yr)	Metacarpal diameter, cross-sectional cortical area (absence of BstB1 restriction site>presence), decrease in radial cortical area with age (absence<presence)		Gong et al. 1999
				Height (6/6>5/5), urinary deoxypyridinoline and pyridinoline (5/5>6/6)		
<i>PTHr1</i>	(AAAG) _n in the P3 promoter	85	Female young Japanese (18–20 yr)		<0.02	Minagawa et al. 2002

Continued

Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
<i>ICF-1</i>	CA repeat upstream of transcription initiation site	274	Japanese postmenopausal women	Total BMD (I-2/I-2<I-1/I-2)	<0.05	Miyao et al. 1998
		116	Healthy white men and women	Serum IGF-I (192/192<other genotypes)	0.03	Rosen & Donahue 1998
OC		25	White men with idiopathic osteoporosis (IOM)	64% homozygosity for 192 allele in the IOM group vs 32% in healthy populations	<0.004	
		300	Postmenopausal Korean women	LS and proximal femur BMD, serum IGF (194/194>194/-, -/-)		Kim et al. 2002c
		97	Healthy white adolescent females (aged 16.9 ± 1.2 yr)	BMD of the humerus (presence of H<absence of H)	0.03	Gustavsson et al. 2000
<i>PPAR-γ</i>	D1S3737	1366	Non-identical twin sisters aged 18–75 yr	BMD, velocity of ultrasound, broadband ultrasound attenuation		Andrew et al. 2002
	C→T transition located in the exon 6	404	Japanese postmenopausal women	Total body BMD (CT, TT<CC)	<0.05	Ogawa et al. 1999
<i>TNFR2</i>	Tri-nucleotide variation in the 3' untranslated region	159	Unrelated individuals	Spine BMD (rarest allele<other)	0.008	Spotila et al. 2000
<i>MGP</i>	CA repeat at the MCP (Matrix Gla protein) gene	460	Elderly Japanese women	BMD at the radial bone (17 CA repeats>no 17 CA repeats)	0.0382	Tsukamoto et al. 2000a
<i>MTHFR</i>	A/V polymorphism	307	Postmenopausal Japanese women	LS BMD (VV<AA) Total body BMD (VV<AA, AV)	0.016 0.03 (VV vs AA), 0.04 (VV vs AA)	Miyao et al. 2000b
<i>p57^{Kip2}</i>	C677T	1748	Healthy postmenopausal Danish women	Lumbar and total hip BMD (TT<CC, CT)		Abrahamsen et al. 2002
	12-bp deletion	154	Postmenopausal Japanese women	BMD [deletion(+)>deletion(-)]	0.021	Urano et al. 2000
<i>ER-β</i>	CA repeat	204	Healthy postmenopausal Japanese women	Lumbar BMD [26 CA repeat (+)>26 CA repeat (-)]	0.027	Ogawa et al. 2000
<i>TNF-α</i>	CA repeat	795	Women with mean age 60 yr (range 29–86 yr)	Ward's triangle BMD [homozygotes of short alleles (<22 repeats)<heterozygotes and homozygotes of large alleles (>22 repeats)]	0.03	Karasik et al. 2002d
	VNTR	104	Postmenopausal osteoporotic white Mediterranean women	Greater lumbar and hip BMD (G allele)	0.0007 (lumbar), 0.02 (hip)	Fontova et al. 2002
<i>OPG</i>	209G→A, 245T→G, haplotype	103	Osteoporotic postmenopausal women	Lumbar BMD	0.005	Arko et al. 2002
	1181G→C in exon 1	511	381 Irish postmenopausal women aged 61.26 ± 8.5, 130 premenopausal women aged 46.3 ± 6.5 yr	Lower lumbar and FN BMD (for C alleles)	0.05 (lumbar), 0.04 (FN)	Wynne et al. 2002

Continued

Table 2 Continued

Gene	Marker locus	n	Population characteristics	Phenotype and effect	P value	Reference
CaSR	CA repeat	472	Postmenopausal Japanese women	BMI and age-adjusted BMD of radial bone (18 repeats<others)	0.0308	Tsukamoto et al. 2000b
	A986S	86	Jewish Israeli patients with idiopathic low BMD (38 premenopausal women and 48 men)	FN BMD (between homozygotes and heterozygotes)	0.002	Eckstein et al. 2002
HLA	HLA-B*07	203	Controls	Frequency of heterozygotes (30% in the patients vs 19.7% in the controls)	0.059	
	HLA-A*24-B*07-DRB1*01	67	Healthy young Japanese women (average age 23.6 ± 2.6 yr)	Peak bone mass: HLA-B*07(+)<HLA-B*07(-); Z score at the LS: HLA-A*24-B*07-DRB1*01 haplotype<other	<0.05	Tsuji et al. 1998
WRN	c.4330T→C	377	Postmenopausal Japanese women	Lumbar and total body BMD (C allele ₊ <C allele ₋)	0.015 (lumbar), 0.042 (total body)	Ogata et al. 2001
CYP17	T→C in the promoter region	333	White men aged 51–84 yr	Serum bioavailable testosterone (C/C>C/T>T/T)	0.019	Zmuda et al. 2001
CYP19	TTTA repeat in intron 4	350	Postmenopausal Italian women (47–76 yr)	Stature, FN cross-sectional area (C/C>T/T)	<0.01	Masi et al. 2001
	TTTA repeat in intron 4	1337	White women over the age of 70 yr	Lumbar BMD (high number of repeats>8–11 repeats)	0.03	Prince et al. 2002
	TTTA repeat	300	Elderly men	Reduced hip BMD [(TTTA) ₁₀ >12]		Gennari et al. 2002
GCCR	N363S [nt 1220 (AAT→AGT)]	216	Elderly persons	Rates of bone loss at LS and Ward's triangle [low repeat (<9)>high repeat(>9)], circulating estradiol (high repeat>low repeat)		Huizenga et al. 1998
IGF-I	CA polymorphism	300	Postmenopausal Korean women	Age, BMI and sex-adjusted BMD (N363S carriers<others)		Kim et al. 2002c
COL1A2	PvuII	91	10–12-yr-old girls	LS and proximal femur BMD, serum IGF (194/194>194/-, -/-)	0.031	Suuriniemi et al. 2002
FRA-1	C101T in exon 1	501	Perimenopausal Scottish women	24-month change in total femur BMD (PP>Pp, pp)	0.007	Albagha et al. 2002
RUNX-2	Synonymous alanine codon polymorphism	495	Women of Geelong Osteoporosis Study	Lumbar BMD (TT<CT, CC)	0.001 (for UD radius)	Vaughan et al. 2002
Klotho gene	G-395A at promoter region, C1818T in exon 4	1187	White women	Higher BMD, especially ultradistal radius (for A allele)	0.001 (for UD radius)	Kawano et al. 2002
		215	Japanese postmenopausal women	Bone density in aged postmenopausal women (≥65 yr)	0.001 (G-395A), 0.010 (C1818T), <0.0001 (haplotype)	
					0.023 (G-395A), 0.035 (C1818T), 0.009 (haplotype)	

FN, femoral neck; LS, lumbar spine; IDDM, insulin-dependent diabetes mellitus.

was the case in the study by Uitterlinden *et al.* (2001) and in situations in which disease, nutrition and environmental factors have an important influence on bone metabolism (Rubin *et al.* 1999).

Studies on *VDR* gene polymorphism have also been extended to clinical settings, including hormone replacement therapy (HRT) or calcium supplementation.

A study in 328 elderly white individuals demonstrated that an increase in BMD in response to calcium intake was present only in those with the bb polymorphism (Kiel *et al.* 1997). However, this advantage for bb individuals could be translated to significantly greater BMD values only if calcium intake exceeded a certain threshold, as suggested by the study in which BMD was greater for bb than for BB or Bb genotypes only in a group in whom calcium intake was greater than 800 mg/day. The opposite genetic effect was found in a sample of prepubertal girls (Ferrari *et al.* 1998a): with increasing dietary calcium intake, BMD accrual increased in only the Bb, and possibly the BB, girls; those with the bb genotype, although having the greatest baseline BMD accrual, remained unaffected, suggesting that high-dosage calcium intake may mask differences in BMD among *VDR* genotypes during the prepubertal period. Given the findings of these two studies, it is possible that the efficiency of calcium absorption declines with age more significantly in individuals of BB genotype than in those with the bb genotype, making the elderly BB individuals become less responsive to calcium supplementation. A lower fractional absorption of Ca^{2+} in BB than in bb individuals had previously been observed in a study of 60 postmenopausal women (Dawson-Hughes *et al.* 1995).

In a study similar to those on calcium supplementation, the effect of vitamin D supplementation on BMD was also tested for its relationship with *VDR* gene polymorphism. Two studies on the increase in BMD in response to vitamin D supplements, one in a Japanese population and another in a Dutch population, identified a positive association of a therapeutic effect of vitamin D with certain *VDR* genotypes (Matsuyama *et al.* 1995, Graafmans *et al.* 1997). The bb genotype was associated with a greater increase in BMD in the Japanese, but the opposite effect was found in the Dutch population, possibly resulting from allelic heterogeneity of the BsmI locus or from different environments for the Japanese and the Dutch populations.

The effect of HRT and bisphosphonate treatment in terms of BMD gain also differs between the *VDR* genotypes. A study in Japanese women receiving HRT for more than 1 year identified different percentage increases in BMD between TaqI genotypes (Kurabayashi *et al.* 1999). The TT genotype benefited from HRT more than did the Tt, with not only a greater increase in BMD, but also a significant decrease in the concentrations of markers of bone resorption. The response to bisphosphonate treatment in combination with calcium supplementation was also found to be modified by the *VDR* genotype in

postmenopausal women with osteoporosis (Marc *et al.* 1999). The lumbar spine BMD increased more rapidly in BB and Bb groups than in the bb group during the 1-year period of treatment, with the bb individuals exhibiting the greatest decrease in osteocalcin concentrations.

Studies on the *VDR* gene polymorphism have been fraught with conflicting results (Table 3). Many well-designed studies could not replicate the allelic associations of the *VDR* gene with bone variables identified in other studies. In the study performed on the largest sample so far (Uitterlinden *et al.* 1996), the polymorphisms for the marker loci of BsmI, ApaI, and TaqI failed to associate with BMD individually. The only positive association, although weak, was found for the haplotype 'bAT', with lower BMD in the femoral neck. Another large-scale association study (Jorgensen *et al.* 1996) also could not establish a relationship between the *VDR* genotypes and BMD at the lumbar spine, hip and forearm or with postmenopausal bone loss. Even among the studies with positive findings, contradictory results exist regarding the effects of the *VDR* alleles on phenotypes. For example, whereas most studies found that the b allele or the bb genotype was associated with greater BMD (Morrison *et al.* 1994, Krall *et al.* 1995), some studies have indicated the opposite (Houston *et al.* 1996, Salamone *et al.* 1996).

One explanation for the negative findings surrounding the study of the *VDR* gene polymorphism (Table 3) may lie in the interaction between gene and environment. Because of the polygenic nature of osteoporosis and bone mass determination, *VDR* polymorphism may underlie only a modest component of the total variation of bone-related traits. As a result, the effect of the polymorphism is often masked by environmental factors. A study by Kiel *et al.* (1997) found that the association of BsmI polymorphism with BMD existed only in a group with a calcium intake of more than 800 mg/day. Similarly, another study on TaqI polymorphism showed that the marker's association with BMD was most significant when daily calcium intake exceeded 684 mg (Rubin *et al.* 1999). Two other studies indicated that the correlation of the *VDR* gene polymorphism with BMD tended to disappear with age and might exist only in the premenopausal (Riggs *et al.* 1995) or even the prepubertal period (Ferrari *et al.* 1998a).

As for the different directions of the genetic effect detected by various studies (i.e. which allele is associated with low or high BMD), either linkage disequilibrium of the *VDR* polymorphic markers with genes related to bone metabolism, or allelic heterogeneity at a certain *VDR* locus for different populations may account for the discrepancies. Theoretically, a recombination between a *VDR* locus and a gene related to bone metabolism, which does not occur in other populations, can lead to a positive association with an opposite effect. Allelic heterogeneity, manifested by different alleles having the same effect (or the same allele having different effects) for different populations, could arise from gene-environment interactions, as

Table 3 Association studies with negative findings

Gene	Marker locus	n	Population characteristics	Phenotype	Reference
VDR	Apal, BsmI, TaqI	86MZ/39DZ	White	BMD	Hustmyer et al. 1994
	Apal, BsmI, TaqI	189	Premenopausal women, white	BMD, bone turnover	Garnero et al. 1995
	Apal, BsmI, TaqI	268	Postmenopausal women, 1–26 yr postmenopausal	Bone turnover, rate of bone loss, BMD	Garnero et al. 1996
		189	Female dizygous twins	Quantitative ultrasound of calcaneus or hip axis length	Arden et al. 1996
	Apal, BsmI, TaqI	101	African-American women >65 yr	BMD, rate of postmenopausal bone loss, bone turnover	Zmuda et al. 1997
	Apal, BsmI, TaqI	84	Type I osteoporotic women (66.6 ± 8.4 yr)	BMD	Vandevyver et al. 1997
	Apal, BsmI, TaqI	103	White women of Mexican descent, postmenopausal	BMD, bone metabolism	McClure et al. 1997
	FokI	174	Premenopausal French women	BMD, calcium, parathyroid hormone, vitamin D, osteocalcin, alkaline phosphatase	Eccleshall et al. 1998
	Apal, BsmI, TaqI	163	Postmenopausal women	BMD	Boschitsch et al. 1996
	Apal, BsmI, TaqI	84	Thai, postmenopausal women	BMD, osteocalcin	Ongphiphadhanakul et al. 1997
	BsmI	81	Old women, >70 yr	FN BMD	Graafmans et al. 1997
	TaqI	48	Men, 27–77 yr	BMD	Francis et al. 1997
	BsmI	50	14 premenopausal	Markers of bone turnover	Rauch et al. 1997
			36 postmenopausal	BMD	
			43.3–62.8 yr	Ultrasound transmission velocity through bone	
	Apal, BsmI, TaqI	268	German women	BMD, BMC, markers of bone turnover	Tsai et al. 1996
			Chinese, 155 men aged 22–88, 113 premenopausal women aged 40–53 yr		
	BsmI	69	Premenopausal white women	BMD	Alahari et al. 1997
		48	Men from southern Europe	BMD	Spotila et al. 1996
		56	Premenopausal women from eastern Europe		
		80	Postmenopausal women from western Europe		
	Apal, BsmI, TaqI	273	Young boys and girls, aged 8.2–16.5 yr	Forearm BMD gain	Gunnes et al. 1997
	BsmI, TaqI	92	49 young women aged 25–35 yr	Intestinal VDR protein concentration, serum 1,25(OH) ₂ D ₃ and radioactive calcium absorption	Kinyamu et al. 1997
	BsmI	38	43 elderly women aged 65–83 yr	Abundance of VDR mRNA in peripheral blood mononuclear cells	Mocharla et al. 1997
	BsmI	9		Level of VDR expression, cellular responsiveness to 1,25(OH) ₂ D ₃ in cultured skin fibroblasts	Gross et al. 1998a
	Apal, BsmI, TaqI	723	Danish women	BMD at LS, hip, and forearm	Jorgensen et al. 1996
		110		Early postmenopausal bone loss (age 51–53 yr)	
		108		Late postmenopausal bone loss (age 63–69 yr)	
		109		Long-term postmenopausal bone loss (age 51–69 yr)	
	FokI	182	Postmenopausal women with sporadic primary HPT	Serum calcium, serum PTH, BMD, parathyroid tumor weight, VDR and PTH mRNA levels, Ca ²⁺ -PTH set points	Correa et al. 1999

Continued

Table 3 Continued

Gene	Marker locus	n	Population characteristics	Phenotype	Reference
VDR	FokI	124	Postmenopausal osteoporotic French women aged 45–90 yr	Age, yr since menopause, height, weight, BMD at LS and FN	Lucotte et al. 1999
	FokI	104	Community-dwelling African-American women aged >65 yr	Hip and calcaneal BMD, calcaneal ultrasound attenuation, hip geometry, biochemical markers of bone turnover, fractional calcium absorption	Zmuda et al. 1999a
	BsmI	191 393 332	Postmenopausal Japanese women Women aged 45–53 yr 177 healthy premenopausal women aged 18.7–56.0 yr, 155 prepubertal girls aged 6.6–11.4 yr	LS baseline BMD BBA, SOS BMD	Kikuchi et al. 1999 Gregg et al. 1999 Ferrari et al. 1998b
	BsmI	172 372	Premenopausal women of white origin Pre- and perimenopausal women	BMD Baseline BMD, change in BMD over 3 yr, bone-related serum markers	Ferrari et al. 1998a Willing et al. 1998
VDR, ER- α , COL1A1, A2	BsmI, ApaI, TaqI	509	272 Chinese women (mean age 75), 237 Chinese men (mean age 73 yr)	BMD	Lau et al. 1999
	BsmI	200	Healthy perimenopausal Danish white women	Lumbar and femoral baseline BMD, bone loss rate, biochemical markers of bone metabolism (bone specific alkaline phosphatase, urinary hydroxyproline, serum osteocalcin)	Hansen et al. 1998
	Cdx BsmI, ER- α PvuII	108 425	Korean postmenopausal women Postmenopausal French-Canadian women, aged 42–85 yr	LS and proximal femur BMD QUS (calcaneal quantitative ultrasound)	Kim et al. 2002b Giguère et al. 2000
		134 239+75	Postmenopausal white women Belgian postmenopausal women, 75 female with osteoarthritis of hip, 239 elderly healthy female controls	BMD No different genotype distributions for the case and control groups, no significant differences in BMD variables were observed for different genotype groups	Willing et al. 1997 Aerssens et al. 1998
VDR, ER- α , COL1A1	Sp1, VDR translation initiation site, osteocalcin gene C/T promoter	261	Pre- and perimenopausal women	BMD and bone turnover	Sowers et al. 1999
	BsmI (VDR), AccB71 (COL1A1), PvuII and XbaI (ER)	135+239	135 hip fracture patients aged 78 $\pm$ 9 yr, 239 controls aged 76 $\pm$ 4 yr	BMD and bone turnover	Aerssens et al. 2000
	Sp1	64 72 38+40	Patients with primary osteoporosis Healthy controls 38 MZ twins, 40 DZ twins, premenopausal white women aged 21–49 yr	BMD BMD at spine and FN	Liden et al. 1998 Hustmyer et al. 1999
	Sp1	314	Women aged 75 yr	Metacarpal cortical index, forearm BMD, tibial SOS, calcaneal SOS, calcaneal BUA, trends toward lower BMD at the hip for Ss and ss	Ashford et al. 2001
	Sp1	133	Postmenopausal women followed for 18 yr	Rate of bone loss; BMD at LS and FN, serum osteocalcin, urinary collagen type I degradation products	Heegaard et al. 2000

Continued

Table 3 Continued

Gene	Marker locus	n	Population characteristics	Phenotype	Reference
COL1A1	Sp1	269	Healthy boys and girls aged 8·2–16·5 yr	Forearm BMD gain in $\pm 3\cdot8$ yr, BMD at forearm, spine, and whole body	Berg <i>et al.</i> 2000
	PvuII, XbaI	426	Italian postmenopausal women	BMD	Gennari <i>et al.</i> 1998
	PvuII	313	Postmenopausal women of white origin in Belgium, of whom 142 suffered a fragility fracture after the age of 50 yr	BMD at LS, FN, proximal forearm. Grip or quadriceps strength. Genotype distribution in women with and without a history of fragility fracture	Vandevyver <i>et al.</i> 1999
ICF-1	PvuII, XbaI	499	Danish postmenopausal women	BMD of hip, spine, lower forearm	Bagger <i>et al.</i> 2000
		101	Postmenopausal women followed for 18 yr	Late postmenopausal bone loss in the hip and spine (over 6 yr), in the lower forearm (over 18 yr)	
	TA repeat	344	144 Japanese females with rheumatoid arthritis (RA) and 200 healthy postmenopausal controls	BMD and radiographs of hands and wrists	Takagi <i>et al.</i> 2000
IL-6		362	295 white, 67 African-American, premenopausal	Spine and femoral neck BMD	Takacs <i>et al.</i> 1999
AR	CAG-repeat polymorphism in exon 1	812	Healthy premenopausal sib pairs of white or African-American origins (20–45 yr)	BMD of the spine and hip	Takacs <i>et al.</i> 2000
IL-1RN	VNTR in intron 2	273	Community-dwelling healthy men (age 71–86 yr)	Hip and forearm BMD, biochemical markers of bone turnover	Van-Pottelbergh <i>et al.</i> 2001b
		286	Postmenopausal women	BMD	Bajnok <i>et al.</i> 2000

MZ, monozygotic; DZ, dizygotic; HPT, hyperparathyroidism; BUA, broadband ultrasound attenuation; SOS, speed of sound.

the same allele may function differently in different environments.

Apart from the above situations, population admixture is another important factor potentially leading to conflicting results in *VDR* association studies (Deng 2001). Population admixture may result in false-positive identification of the association of a polymorphism with a complex trait (Chakraborty & Smouse 1988, Deng & Chen 2000b, Deng *et al.* 2001a). It could also mask, change or reverse true genetic effects of genes underlying complex traits, as demonstrated by Deng (2001) through a simple one-locus population genetics model.

The *VDR* gene maps to chromosome 12q13-14 and contains at least 11 exons (Haussler *et al.* 1998). The BsmI polymorphism is located between exon VIII and the 3' non-coding region. The TaqI restriction site, defining the TaqI polymorphism, is located within exon IX. Between these two polymorphisms within intron VIII is the Apal polymorphic locus. A strong linkage disequilibrium exists between the BsmI and TaqI polymorphism such that the B allele is usually associated with the t allele and the BB genotype is almost equivalent to the tt genotype. In contrast, the Apal RFLP is not strongly associated with the BsmI and TaqI RFLPs. In addition to the three loci, another polymorphism, the start codon polymorphism (SCP), as identified by the FokI polymorphism, is located at the translation initiation site in exon II. Unlike the first three polymorphisms, which do not result in amino acid changes, the SCP causes a change in *VDR* structure, making the F allele *VDR* three amino acids shorter than those of the f allele. Such a difference in *VDR* primary structure could lead to altered receptor function (Arai *et al.* 1997).

Although the BsmI locus is intronic, a number of mechanisms have been invoked to explain how this polymorphism might influence expression of the *VDR* gene and alter BMD. One mechanism is disruption of a splice site for *VDR* mRNA transcription. Another mechanism involves changes in mRNA stability or in the intronic regulation element (Cheng *et al.* 1994, Nesic *et al.* 1993). As suggested by Morrison *et al.* (1994), the B allele was associated with greater levels of *VDR* through enhanced transcriptional activity or greater mRNA stability. However, the difference either in *VDR* mRNA stability or in *VDR* mRNA levels between *VDR* genotypes/alleles could not be detected in other studies (Mocharla *et al.* 1997, Verbeek *et al.* 1997). Attempts to relate *VDR* genotype with *VDR* concentrations in duodenal mucosa also failed (Barger-Lux *et al.* 1995). To explore further the effects of BsmI polymorphism on the properties of the *VDR* and on cellular responsiveness to $1,25(\text{OH})_2\text{D}_3$, a study was conducted on cultured skin fibroblasts from BB, Bb and bb individuals (Gross *et al.* 1998a). Among the three genotypes, no significant differences were found in *VDR* abundance, affinity for $[3\text{H}]1,25(\text{OH})_2\text{D}_3$, *VDR* mRNA levels and fibroblast

responsiveness to $1,25(\text{OH})_2\text{D}_3$. Given the above findings, it is unlikely that the *VDR* BsmI (or TaqI) polymorphism is functionally related to variation in BMD; rather, it may be only a marker in strong linkage disequilibrium with a nearby functional mutation that underlies variations in bone mass. Other possibilities remain open to investigation; for example, functional variations in the *VDR* that are caused by the BsmI polymorphism might be detectable only in specific tissues, such as bone or intestine.

As the SCP (the FokI RFLP) leads to production of *VDR* proteins that differ in length by 3 amino acids, the polymorphism may represent a mechanism resulting in variation in BMD. Using transfected HeLa cells, Arai *et al.* (1997) detected significantly greater vitamin D-dependent transcriptional activation for the F allele (short type) *VDR* than for the f allele (long type) *VDR*. However, another study on the two types of *VDR* expressed in COS-7 cells failed to confirm a functional relevance of the FokI SCP. There was no difference detected between the two isoforms of the *VDR* with respect to affinity for $[3\text{H}]1,25(\text{OH})_2\text{D}_3$, ability to bind DNA and ability to transactivate target genes (Gross *et al.* 1998b). Moreover, in human fibroblasts of the three genotypes – FF, Ff and ff – the sensitivity to $1,25(\text{OH})_2\text{D}_3$ with respect to the induction of 24-hydroxylase mRNA was also generally the same (Gross *et al.* 1998b). The above findings should be interpreted with caution. Current approaches to the detection of small differences in *VDR* transactivation activity are limited by low sensitivity; as a result, physiologically important but small functional differences between the F and f *VDR* isoforms could be undetected. In addition, differential function caused by the FokI SCP may manifest itself only in some specific tissues, such as bone and intestine. Conversely, the lack of functional significance of the FokI SCP of the *VDR* gene, as indicated in the study by Gross *et al.* (1998b), supports the hypothesis that this polymorphism, as in the case of the BsmI polymorphism, could be only a marker in linkage disequilibrium with a nearby gene that is functionally related to bone mass regulation.

Recently, a novel polymorphism in the Cdx-2 binding site of the *VDR* gene promoter region was identified (Yamamoto *et al.* 1999, Arai *et al.* 2001). The polymorphism was defined by an A→G change at the –3731nt relative to the transcription start site of the *VDR* gene. Functional analysis of the polymorphic region, which is also called the human *VDR*–sucrase–isomaltase footprint 1 (hVD–SIF1) sequence, was performed using an intestinal cell line as a model system (Yamamoto *et al.* 1999). It was found that, by binding to the hVD–SIF1 sequence, Cdx-2 was able to activate *VDR* gene transcription. Mutations of the sequence suppressed transactivation activity. Insertion of a fragment containing the hVD–SIF1 sequence upstream of the thymidine kinase promoter of a herpes simplex virus enhanced its transcription activity. These findings suggest the important role of the Cdx-2

binding site in regulation of intestine-specific *VDR* gene expression.

To test the significance of the *Cdx* polymorphism in human population, an association study was conducted in 261 Japanese women (Arai *et al.* 2001). The BMD at the lumbar spine of the *Cdx*-A homozygotes was significantly greater than that in the *Cdx*-G homozygotes. Functional analysis of the two genotypes showed markedly decreased transcriptional and *Cdx*-2 binding activities for the *Cdx*-G homozygotes as compared with the *Cdx*-A homozygotes. Given the close relationship between the *Cdx* polymorphism and the intestinal expression of the *VDR*, it is speculated that the polymorphism regulates BMD through intestinal *VDR* content, an important factor contributing to the development of osteoporosis. As estrogen was able to upregulate the expression of *VDR* in the duodenal mucosa (Liel *et al.* 1999), the effect of the *Cdx* polymorphism on BMD may be influenced by estrogen status, as indicated by the finding of the association of the *Cdx* polymorphism with BMD only in postmenopausal, and not premenopausal, women (Arai *et al.* 2001). The significance of the polymorphism for bone has yet to be validated. A more recent study in a group of Korean women was unable to replicate the association of the *Cdx* polymorphism with BMD (Kim *et al.* 2002b). Furthermore, the study failed to detect a significant difference in prevalence of the polymorphism between patients with osteoporosis and normal controls.

COLIA1 gene

Mutations in the *COLIA1* gene cause osteogenesis imperfecta, a disease characterized by moderate to severe bone fragility (Byers 1990). The similar bone brittleness observed in osteogenesis imperfecta and in osteoporosis makes it interesting to speculate that osteoporosis may also be caused by mutations of the *COLIA1* gene. Screening the transcriptional control regions of the *COLIA1* gene identified a G→T substitution at the first base of a consensus site in the first intron for the transcriptional factor, Sp1 (Chu *et al.* 1985, Rossouw *et al.* 1987). The polymorphism was thus named the Sp1 polymorphism. A population-based study of this polymorphism detected an association with BMD in two groups of British women (Grant *et al.* 1996): BMD was greater for the G/G (SS) genotype than for the G/T (Ss) and T/T (ss) genotypes. Over-representation of the unfavorable Ss and ss genotypes was found in patients with osteoporosis and fractures, conferring a relative risk of 2.97 for vertebral fracture in individuals carrying the s allele.

Functional analysis of the Sp1 polymorphism by Mann *et al.* (2001) demonstrated functional differences between the two alleles. The s allele had greater affinity for Sp1 protein than did the S allele. In the Ss heterozygotes, the RNA transcripts derived from the s allele were three times more abundant than those from the S allele, suggesting

allele-specific transcription or a different splicing process for the two alleles. Increased ratios of $\alpha 1(I)$ to $\alpha 2(I)$ protein in collagen and increased ratio of *COLIA1* to *COLIA2* mRNA were detected using osteoblasts cultured from the Ss heterozygotes. This is consistent with the possibility of an increased production of $\alpha 1(I)_3$, which was reported by several investigators to be responsible for impaired bone strength in osteogenesis imperfecta (Deak *et al.* 1985, Chipman *et al.* 1993, Saban *et al.* 1996, McBride *et al.* 1998). Although the presence of $\alpha 1(I)_3$ could not be determined, in individuals carrying the s allele the study did reveal a decrease in bone strength that was independent of BMD, suggesting that decreased bone strength may be caused more by poor organic (e.g. collagen) content of bone than by decreased bone mineral content.

The positive association of the Sp1 polymorphism with bone phenotypes has been confirmed in several studies. Two large-scale studies identified the relationship of the Sp1 genotype with BMD in 1778 postmenopausal Dutch women and in 715 peri- and postmenopausal Italian women (Uitterlinden *et al.* 1998, Braga *et al.* 2000), with BMD difference between the Sp1 genotypes becoming more significant with increasing age in the first study. The association with BMD has also been identified in pre-pubertal girls (Sainz *et al.* 1999) and elderly men (Van-Pottelbergh *et al.* 2001a). The Sp1 polymorphism may also influence bone loss and bone turnover, with the ss genotype associated with greater bone loss (Harris *et al.* 2000, MacDonald *et al.* 2001) and increased levels of bone turnover (Keen *et al.* 1999). Notably, the association with bone loss could be modulated by dietary calcium intake, as was reported by Brown *et al.* (2001), who found that lumbar spine bone loss was greater in the ss and Ss genotypes than in the SS genotype for the lowest tertile of calcium intake, but that the opposite effect (i.e. SS individuals lost more bone than the Ss and the ss individuals) was true for the greatest calcium intake. The Sp1 polymorphism has been found to be associated with response to bisphosphonate treatment in postmenopausal women with osteopenia (Qureshi *et al.* 2002). However, the association was significant only at the femoral neck, suggesting a site-specific effect of the polymorphism. Over-representation of the s allele or the ss genotype in patients with osteoporosis or fractures has been reported in many studies (Table 4). The s allele or ss genotype confers a risk for osteoporosis or fractures with an odds ratio ranging from 2.1 (Weichetova *et al.* 2000, Mezquita-Raya *et al.* 2002) to 11.83 (Langdahl *et al.* 1998). The relevance of the polymorphism to risk of fracture could probably be mediated through variations in bone geometry, as indicated by the findings of a recent study in which the femoral neck-shaft angle was wider in Ss/ss than in the SS individuals, which led to a greater risk of fracture for the former group (Qureshi *et al.* 2001).

Two new single nucleotide polymorphisms (SNPs), -1663 indelT and -1997 G/T, were identified within

Table 4 Risk factors contributing to osteoporosis and other related diseases

Gene	Risk factor	Risk	Odds ratio (95% CI)	P value	Sample size and character	Reference
VDR	BB genotype	Hip fracture (BB>bb)			White women 43–69 yr 54 proximal femur (hip) fractures	Feskanich et al. 1998
	bb genotype	Primary HPT	bb 60.0% HPT patients bb 33.3% postmenopausal female controls	0.001	90 postmenopausal women with primary HPT	Carling et al. 1995
	TT genotype	Severity of osteophytosis Presence of disc narrowing Presence of osteophytosis	TT vs tt: 0.41 (0.17 to 0.97) TT vs tt: 0.45 (0.20 to 0.99) TT vs tt: 0.47 (0.19 to 1.16)		110 men and 172 women over 60 yr	Jones et al. 1998
	allele T	Knee osteoarthritis	T vs t: 2.82 (1.16 to 6.85)	0.02	351 postmenopausal women (aged 45 to 64 yr)	Keen et al. 1997b
	baT alleles	Sporadic primary HPT	TT: 3.4 (1.7 to 6.6) aa: 2.6 (1.3 to 5.3) bb: 3.3 (1.7 to 6.4)	TT: 0.0004 aa: 0.006 bb: 0.0006	206 white patients	Carling et al. 1997a
	baT alleles	Primary HPT, homozygosity for b, a, T was over-represented in pHPT		<0.01–0.05	66 postmenopausal women with primary HPT and 66 age-matched female controls	Carling et al. 1998b
COL1A1	ff genotype	Vertebral fracture	2.58 (1.36 to 4.91)	0.003	Postmenopausal women of Italian origin, 164 osteoporotic, 117 osteopenic, 119 normal	Gennari et al. 1999
	baT haplotype	Fractures	2.6 (1.4 to 5.0) for homozygous carriers of baT, 1.8 (1.0 to 3.3) for baT heterozygous carriers		1004 postmenopausal women	Uitterlinden et al. 2001
	baT haplotype	Radiographic osteoarthritis at the knee	2.27 (1.46 to 3.52)			
	ff genotype	Osteoporosis	2.8 (ff vs Ff)	<0.05	846 old people aged 68.5 ± 6.9 yr	Uitterlinden et al. 1997
	s allele	Fracture prevalence	1.95 (1.01 to 3.78)	0.04	163 postmenopausal women in Taiwan	Chen et al. 2002
	ss genotype	Vertebral fracture Osteoporosis in men Osteoporosis in women Osteoporotic fracture Vertebral fracture	11.83 (2.64 to 52.97) 2.04 1.37 0.028 2.1 (1 to 4.3)		185 healthy women (mean ± SD age 54.3 ± 4.6 yr) 375 osteoporotic vertebral fracture patients and normal controls	Keen et al. 1999
	T allele			0.029		Langdahl et al. 1998
	TT genotype	Fracture prevalence	4.8 (TT vs GG)		Postmenopausal Spanish women, 43 with vertebral fracture, 101 without fracture Postmenopausal women, 98 non-osteoporotic, 139 osteoporotic without fracture, 82 osteoporotic with fracture	Mezquita-Raya et al. 2002 Bernad et al. 2002

Continued

Table 4 Continued

Gene	Risk factor	Risk	Odds ratio (95% CI)	P value	Sample size and character	Reference
COL1A1	ss genotype	Fracture prevalence	1.25 (1.09 to 1.45) (Ss vs SS); 1.68 (1.35 to 2.10) (ss vs SS); 1.35 (1.04 to 1.75) (ss vs Ss) 2.59 (1.23 to 5.45)		Meta-analysis of 13 studies with 3641 participants	Efstathiadou et al. 2001b
	s allele	Osteoporotic fractures in 4.88 ± 0.03 yr			185 postmenopausal women	McGuigan et al. 2001
	s allele	Wrist fracture	2.1 (1.2 to 3.8) per copy of s allele; 2.0 (1.1 to 3.8) Ss vs SS; 2.8 (0.5 to 14.6) (ss vs SS)		126 Czech postmenopausal women with low bone mass and wrist fractures; 126 Czech postmenopausal women with low bone mass without fractures	Weichetova et al. 2000
	Sp1	Vertebral fracture	2.26 (1.09 to 4.69)		93 patients with vertebral fracture and 88 age-matched controls	McGuigan et al. 2000
ER-α	s allele and Ss genotype	Idiopathic osteoporosis	29% vs 11% (s frequency for patients vs controls); 46% vs 18% (Ss frequency for patients vs controls)	0.003	35 male patients with idiopathic osteoporosis and 60 healthy controls	Peris et al. 2000
	ss genotype	Fractures in men	8.56 (2.32 to 31.5)		156 men, aged 64 ± 9 yr	Alvarez-Hernandez et al. 2002
	G/T and T/T genotype of Sp1	Fractures	Over-representation in fracture cases	0.04	149 postmenopausal Scottish women with or without vertebral fractures	McGuigan et al. 2002
	Sp1/- 1663delT haplotype	Surgical menopause (especially hysterectomy because of fibroids or menorrhagia)	6.1 (2.61 to 14.1)		900 postmenopausal women, aged 55–80 yr	Weel et al. 1999
	PvuII PP vs pp	Osteoporotic fractures	2.4 (1.5 to 3.8) (PP vs pp)		160 women and 30 men with vertebral fractures, 124 women and 64 men as controls	Langdahl et al. 2000b
TCF-β1	TA repeat in promoter region	Vertebral fracture	2.64 (1.61 to 4.34) (with 11–18 repeats)		610 postmenopausal women	Becherini et al. 2000
	TA repeat at the 5'	Osteoporosis	2.9 (1.56 to 5.72) (with TA repeats <15) 2.7 (1.49 to 4.76) per A allele		228 postmenopausal Thai women with (n = 106) and without (n = 122) osteoporosis	Ongphiphadhanakul 2001b
	G2014A SNP polymorphism in exon 8	Fractures	0.6 (0.47 to 0.93) (XX vs other)		1591 women from 30 studies (meta-analysis)	Ioannidis et al. 2002
	XbaI	Osteoporosis	Frequency of TT in osteoporosis patients > controls		286 individuals with osteoporosis and 170 normal controls	Yamada et al. 2001

Continued

Table 4 Continued

Gene	Risk factor	Risk	Odds ratio (95% CI)	P value	Sample size and character	Reference
OC	Osteocalcin gene HindIII	Osteopenia	5.74 (HH vs hh) 1.59 (Hh vs hh)	<0.05	160 postmenopausal Japanese women (age 48–80 yr)	Dohi et al. 1998
	Osteocalcin gene HindIII	Osteopenia	4.5 (presence of H)	0.03	97 healthy white adolescent females (aged 16.9 ± 1.2 yr)	Gustavsson et al. 2000
	Osteocalcin gene HindIII	Osteoporosis	6.4 (HH vs hh) 1.2 (Hh vs hh)	<0.05	Chinese postmenopausal women in Taiwan	Chen et al. 2001c
ApoE	Apolipoprotein E (APOE*4 allele)	Hip fracture, wrist fracture	Hip fracture: 1.90 (1.05 to 3.41) Wrist fracture: 1.67 (1.01 to 2.77)		1750 women, age ≥ 65 yr	Cauley et al. 1999
	Apolipoprotein E	Bone fracture	Greater percentage of patients with E3/4 and E4/4 than of patients with E2/3, E2/2 had a history of bone fracture	<0.005	219 hemodialysis patients	Kohlmeier et al. 1998
IL-1ra	86 base pair VNTR, A1A1, A1A3 genotypes	Osteoporosis	1.68 (1.01 to 2.77)		389 osteoporotic patients with vertebral fractures and normal controls	Langdahl et al. 2000c
OPG	A163G in promoter region	Fracture	Allele G more common among fracture patients. OR: 1.44 (1.00 to 2.08)	<0.05	268 osteoporotic patients and 327 normal controls	Langdahl et al. 2002
IGF-I	T245G in promoter region		Allele G more common among osteoporotic patients OR: 2.00 (1.10 to 3.62)	<0.02		
	G1181C in exon 1		Genotype CC less common among fracture patients	<0.01		
	Promoter polymorphism	Frailty fracture	1.4 (1.0 to 1.9) (non-carriers of 192-bp vs carriers) 2.2 (1.2 to 4.2)		905 subjects from Rotterdam study	Rivadeneira et al. 2002
CYP19		Fractures at proximal humerus	(non-carriers vs homozygotes), 1.8 (1.1 to 2.9) (heterozygotes vs homozygotes)			
	TTTA repeat in intron 4	Spine fractures	4.1 (1.19 to 13.87) (shorter vs longer TTTA repeats)	0.02	350 postmenopausal Italian women	Masi et al. 2001

HPT, hyperparathyroidism; OR, odds ratio.

the *COL1A1* promoter region (Garcia-Giralt *et al.* 2002). When tested in 256 postmenopausal women, the -1997 G/T SNP showed association with BMD at the lumbar spine and femoral neck. There was no association with BMD for the -1663 indelT SNP, but the marker was in strong linkage disequilibrium with the Sp1 polymorphism, suggesting that the two polymorphisms may be functionally related to each other. Functional assays found that the regions containing these two SNPs may be the binding sites of primary osteoblast nuclear proteins. There might be interactions of the two SNPs with the Sp1 polymorphism, because individuals heterozygous at all of the three loci had the greatest BMD. Further studies are necessary to delineate the mechanisms underlying the effects of these SNPs on BMD variation and to test their clinical relevance for general populations.

Association studies of the *COL1A1* gene have also been inconsistent, as in the case of the *VDR* gene polymorphisms (Table 3). A series of studies could not establish an association of the Sp1 polymorphism with BMD (Aerssens *et al.* 1998, 2000, Sowers *et al.* 1999, Berg *et al.* 2000, Ashford *et al.* 2001), bone turnover (Sowers *et al.* 1999, Aerssens *et al.* 2000, Heegaard *et al.* 2000), bone loss (Heegaard *et al.* 2000), bone accrual (Berg *et al.* 2000) and other bone phenotypes, such as calcaneal broadband ultrasound attenuation (Ashford *et al.* 2001). Furthermore, one of these studies (Aerssens *et al.* 1998) failed to detect differences in genotype prevalence between osteoporotic patients and control groups.

In an attempt to clarify the inconsistencies, two meta-analyses were conducted. The first was performed using the data from 16 studies published between October 1996 and October 2000 (Mann *et al.* 2001). According to the meta-analysis, there is a significant association between possession of the *COL1A1* s allele and low BMD, with a clear allele dose-effect at both the lumbar spine and femoral neck. This association was also found to be independent of body mass index (BMI). Another meta-analysis was conducted on data from 13 studies with 3641 participants (Efsthadiadou *et al.* 2001b). A dose-response relationship of the Sp1 polymorphism with fracture prevalence was identified. It was also found that the effect of the polymorphism was greater for vertebral fractures and tended to disappear when the individual was older than 65 years.

Results from these two meta-analyses, together with the data from functional studies (Mann *et al.* 2001), confirmed the importance of the Sp1 polymorphism in bone metabolism. An investigation of the polymorphism on ethnically different samples estimated that from 1.7% to 6.8% of the difference in hip fracture rates between Asian and white populations may be attributed to difference in prevalence of the s allele between the two populations (Beavan *et al.* 1998). However, because of the moderate effect of the Sp1 polymorphism on bone metabolism, the relationship of the polymorphism with bone phenotypes can easily be

skewed, or even masked, by such factors as race, age, estrogen status and nutrition. Hence the predictive power of the Sp1 polymorphism for osteoporosis and fractures is intrinsically limited. Increasing genotype resolution by haplotyping including other polymorphisms, controlling potential heterogeneity by subdividing the study population or by using a family-based study design (Spielman *et al.* 1993, Deng *et al.* 2002a), and identifying more causative polymorphisms using SNP markers and linkage disequilibrium mapping are among some of the alternative approaches to finding the true functional mutations.

ER-α gene

The *ER-α* gene is located on chromosome 6q25-27. It comprises eight exons and spans more than 140 kb. Several polymorphisms for the gene have been studied. Among them, the T→C and the A→G in intron 1 and the TA repeat in the promoter region are those most frequently studied. Novel polymorphisms are continuously being identified and tested for association; recent examples include codon 325 (CCC→CCG) (Jurada *et al.* 2001), T262C of exon 1 (Ongphiphadhanakul *et al.* 2001a), G2014A of exon 8 (Ongphiphadhanakul *et al.* 2001b), and G261C of exon 1 (Langdahl *et al.* 2000b).

The TA repeat polymorphism is located within the promoter region 1174 bp upstream from exon 1. This polymorphism was speculated to affect bone mass by changing the production or stability of mRNA, resulting in changes in ER number. The hypothesis has yet to be confirmed by functional studies. The association of the polymorphism with BMD was first reported in a study on 144 postmenopausal Japanese women (Sano *et al.* 1995). The allele C, having 12 dinucleotide repeats of TA, was found to be associated with lower spine and total body BMD and greater concentrations of markers of bone turnover, including serum osteocalcin, urinary pyridinoline and deoxypyridinoline. The finding was supported by another study that identified the effect of the TA polymorphism on total body calcium (Sowers *et al.* 1999). Three more recent studies also confirmed the correlation of the TA repeat number with BMD values in North European (Langdahl *et al.* 2000b), Mediterranean (Becherini *et al.* 2000) and Southern Chinese (Chen *et al.* 2001b) populations. In the earlier two studies (Becherini *et al.* 2000, Langdahl *et al.* 2000b), the TA repeat number was positively correlated with lumbar or hip BMD – larger numbers of repeats tended to associate with higher BMD values – but the opposite effect was found in the Chinese population (Chen *et al.* 2001b). Correspondingly, a high risk for fracture or osteoporosis was conferred by low-range repeats (11–18) in the European populations (Becherini *et al.* 2000, Langdahl *et al.* 2000b) and a high number of repeats (TA ≥ 20) in the Chinese population (Chen *et al.* 2001b).

Two polymorphisms, T→C and A→G, in the first intron have also been extensively studied. They are located respectively ~400 bp and ~350 bp upstream of exon 2. The functional basis for the influence of the two intronic polymorphisms, defined as PvuII and XbaI, on bone mass variation remains unclear. A possible explanation is that the two polymorphisms may be associated with bone phenotypes via their strong linkage disequilibrium with nearby functional loci, such as the TA repeat in the promoter region, the insulin-like growth factor I gene (*IGF-I*), and the PTH-related peptide gene (*PTHr*).

Association of the PvuII and the XbaI polymorphisms with BMD has been detected in many studies (Table 2). In general, the XX and pp genotypes (or the X and p alleles) were associated with greater BMD as compared with the xx and PP genotypes (or the x and P alleles) (Kobayashi *et al.* 1996, 2002, Mizunuma *et al.* 1997, Willing *et al.* 1998, Albagha *et al.* 2001). The opposite effect was found in Thai and Korean women, Thai men and Finnish prepubertal girls (Ongphiphadhanakul *et al.* 1998a, Kim *et al.* 2001, Suuriniemi *et al.* 2002). A large-scale meta-analysis of 30 studies confirmed both the significance and the direction (XX>other genotypes) of the effect of the XbaI polymorphism on BMD of the total body, femoral neck and lumbar spine (Ioannidis *et al.* 2002). However, the meta-analysis could not establish an association between the PvuII polymorphism and BMD.

Notably, two independent Japanese studies found that the individuals with greater bone mass (with the XX and the pp genotypes) also had greater bone turnover and significant bone loss, suggesting that the BMD differentiation between the XbaI or PvuII genotypes tends to decrease with age and disappear eventually through the late postmenopausal period, if there is no intervention. This may help explain why most of the inconsistent results concerning *ER-α* gene polymorphisms came from studies on PvuII and XbaI polymorphisms (Table 3), as exemplified by studies in 426 Italian postmenopausal women and in 248 Korean postmenopausal women (Han *et al.* 1997, Gennari *et al.* 1998). Potentially, it may also undermine the value of these two polymorphisms as predictive factors for osteoporosis and fractures.

Other genetic polymorphisms

Since the recognition of the *VDR* gene polymorphism as one of the important parameters predictive of bone variables, the candidate-gene approach has become the mainstay of the methodology for genetic study of osteoporosis. A host of novel polymorphisms have been identified and related to variation in bone mass and risk for osteoporotic fractures (Table 1). Most of the polymorphisms are from the genes encoding the important pathways of bone and mineral homeostasis and metabolism. Representatives of these genes include those for transforming growth factor-β1 (*TGF-β1*), interleukin-6 (*IL-6*), *IGF-I*, calci-

tonin (*CT*), calcitonin receptor (*CTR*) and interleukin-1 receptor antagonist (*IL-1ra*) (Table 2).

TGF-β1 has been implicated as a potential mediator coupling bone resorption and formation. The *TGF-β1* gene has seven exons, of which exons 5–7 encode the active TGF-β1. Several polymorphisms of the gene have been studied. In one study of a large sample involving 1706 female dizygotic twins, the T→C polymorphism of intron 5 was associated with femoral neck BMD (Keen *et al.* 2001). A study on the 509C→T polymorphism in 625 postmenopausal Japanese women detected its relationship with lumbar spine and total body BMD (Yamada *et al.* 2001). Two independent studies of the T→C polymorphism of the signal sequence region yielded different results in terms of the effect of the polymorphism on BMD. A study in a Japanese population identified that the CC genotype was associated with greater BMD and lower frequency of vertebral fracture as compared with the TT genotype (Yamada *et al.* 1998), but another one, in a German population, detected that the TT genotype instead was the one associated with greater BMD and less bone loss (Hinke *et al.* 2001). Two other polymorphisms, the 713–8 delC and the T→C of exon 1, were also related to BMD, bone turnover, bone loss and response to vitamin D and HRT (Langdahl *et al.* 1997, Bertoldo *et al.* 2000, Yamada *et al.* 2000).

IL-6, as a pleiotropic cytokine, has important effects on osteoclast differentiation and function. It was found to mediate estrogen-deficiency-related bone loss in rodents (Manolagas & Jilka 1995) and its expression was upregulated in bone tissue from osteoporotic patients (Ralston 1994). A few studies have demonstrated the relationship of several polymorphisms of the *IL-6* gene with bone phenotypes, including the variable number tandem repeat (VNTR) polymorphism in the 3' flank region of the gene (Murray *et al.* 1997), the CA repeat polymorphism (Tsukamoto *et al.* 1999), the BsrBI endonuclease C/G polymorphism at (nt) –634 of the promoter region (Ota *et al.* 2001), and the –174 G→C polymorphism (Ferrari *et al.* 2001). The latter polymorphism was also associated with bone resorption (Ferrari *et al.* 2001), consistent with the osteoclast-dependent regulatory mechanism of IL-6. A functional study of the polymorphism using transfected Hela cells revealed significantly lower expression of IL-6 for the –174 C than for the 174 G construct (Fishman *et al.* 1998). That difference in expression between the two alleles persisted upon stimulation of proinflammatory mediators such as IL-1. In addition, the allele C was also found to be associated with lower plasma concentrations of IL-6 in 102 healthy individuals (Fishman *et al.* 1998). It is speculated that transcriptional modulation by this polymorphism results from its potential influence on the binding of the glucocorticoid receptor to a nearby region of DNA (Fishman *et al.* 1998). It is also possible that the G→C change at –174 creates a potential binding site for the transcription factor nuclear factor-1 (Liu *et al.* 1997,

Fishman *et al.* 1998). These results implicated the -174 G/C polymorphism as the key factor regulating IL-6-mediated inflammatory processes, which are important in the pathogenesis of diseases such as osteoporosis, arthritis and atherosclerosis. The association of the BsrBI polymorphism with BMD (Ota *et al.* 2001) could not be replicated in a sample of Chinese nuclear families using the transmission disequilibrium test approach (SF Lei, YM Li, FY Deng, MX Li, YJ Qin, Q Zhou, XD Chen, MY Liu, YZ Liu *et al.*, unpublished observations). Nevertheless SF Lei *et al.* (unpublished observations) did establish the linkage of the BsrBI locus with spine BMD, suggesting that the polymorphism may still link to a QTL underlying the variation in BMD in the Chinese population.

IGF-I is produced by osteoblasts and stimulates skeletal growth through its regulation of cell proliferation, differentiation, DNA synthesis, and collagen and non-collagen protein (Canalis 1980, Mohan & Baylink 1991, Canalis *et al.* 1998, Hock *et al.* 1998). It also functions as a mediator of a series of hormones involved in bone metabolism, such as growth hormone, estrogen and PTH (Gray *et al.* 1989, McCarthy *et al.* 1989, Ernst & Rodan 1991, Inzucchi & Robbins 1994, Watson *et al.* 1995). A CA repeat polymorphism upstream of the transcriptional initiation site of the gene has been investigated in several studies (Miyao *et al.* 1998, Rosen & Donahue 1998, Kim *et al.* 2002c). Associations of the polymorphism with both BMD and serum IGF-I concentrations were identified. As serum IGF-I concentrations were correlated with bone mass in both inbred strains of mice (Beamer *et al.* 1996, Rosen *et al.* 1997) and humans (Kurland *et al.* 1997, 1998) and corresponded to the skeletal content of IGF-I (Beamer *et al.* 1996, Rosen *et al.* 1997), the difference in BMD between IGF-I genotypes may be mediated via variations in serum IGF-I. However, the causative relationship between serum IGF-I and bone mass requires further investigation, as serum IGF binding protein-5 was also found to be positively associated with femoral neck BMD in females (Karasik *et al.* 2002c).

Calcitonin is a polypeptide hormone secreted by parafollicular cells of the thyroid gland. It inhibits osteoclastic bone resorption and stimulates urinary calcium excretion. The human CTR belongs to a family of G-protein-coupled receptors with seven spanning domains. Polymorphisms of both the *CT* and the *CTR* genes were studied for their relationship with bone mass. In a study in 663 postmenopausal and 52 perimenopausal Italian women, the AluI restriction enzyme polymorphism of the *CTR* gene was associated with spine BMD, with greater effect in the younger subset (Braga *et al.* 2000), suggesting that the polymorphism may have more influence on the peak bone mass than on age-related bone loss. Another two polymorphisms of the *CTR* gene, the TaqI polymorphism and the T→C polymorphism, were also found to be related to lumbar and femoral neck BMD (Masi *et al.* 1998, Taboulet *et al.* 1998). The CA repeat polymorphism

of the *CT* gene was correlated with lumbar BMD in a group of Japanese postmenopausal women, with the 10-repeat allele being associated with lower BMD values (Miyao *et al.* 2000a).

IL-1 induces bone resorption by activating osteoclasts via osteoblasts and by inhibiting apoptosis of osteoclasts. IL-1ra competes with both IL-1 α and IL-1 β , the two isoforms of IL-1, for binding with IL-1 receptors without agonist effects. Estrogen-mediated production of IL-1 and IL-1ra represents an important mechanism of postmenopausal bone loss (Pacifi *et al.* 1998). The human *IL-1ra* gene maps to chromosome 2q13-14 and consists of four exons (Lennard *et al.* 1992). A VNTR of 86 bp repeats within intron 2 of the gene was tested for association with bone variables in several studies. Significant association of the VNTR polymorphism with rates of change in spinal bone mass, spine bone loss (Keen *et al.* 1998), and lumbar and hip BMD (Langdahl *et al.* 2000c, Fontova *et al.* 2002) was identified.

The polymorphisms of other genes related to bone metabolism also have been extensively investigated (Table 2). A microsatellite marker, D1S3737, of the osteocalcin gene was studied in 1366 non-identical twin sisters (Andrew *et al.* 2002). Allele 10 of the marker was found to be negatively associated with BMD, velocity of ultrasound and broadband ultrasound attenuation using a multivariate model-fitting approach. Another polymorphism of the gene, the HindIII polymorphism, was related to humerus BMD in adolescent females; those with the H allele had significantly lower BMD than those without the allele (Gustavsson *et al.* 2000). The BstBI polymorphism of the *PTH* gene was associated with BMD and bone turnover markers in 383 postmenopausal Japanese women (Hosoi *et al.* 1999). It was also able to influence such important indices of bone geometry as metacarpal diameter, cross-sectional cortical area and age-related decrease in radial cortical area (Gong *et al.* 1999).

A series of genes have been newly recognized as related to variation in BMD (Tables 1 and 2) (Dickson *et al.* 1994, Shiraki *et al.* 1997, Huizenga *et al.* 1998, Tsuji *et al.* 1998, Zmuda *et al.* 1998, 2001, Ogawa *et al.* 1999, 2000, Sowers *et al.* 1999, Miyao *et al.* 2000b, Salamone *et al.* 2000, Spotila *et al.* 2000, Tsukamoto *et al.* 2000a,b, Urano *et al.* 2000, Masi *et al.* 2001, Ogata *et al.* 2001, Albagha *et al.* 2002, Arko *et al.* 2002, Eckstein *et al.* 2002, Fontova *et al.* 2002, Gennari *et al.* 2002, Karasik *et al.* 2002d, Kawano *et al.* 2002, Minagawa *et al.* 2002, Prince *et al.* 2002, Suuriniemi *et al.* 2002, Vaughan *et al.* 2002, Wynne *et al.* 2002). For most of these genes, these were the first reports of an association with BMD and there is a need to repeat the findings in independent studies. There has been preliminary confirmation of the relevance of several genes to variation in BMD. Two studies on the *ER- β* gene both detected association of the CA repeat polymorphism with BMD, one at the lumbar spine (Ogawa *et al.* 2000) and another at Ward's triangle (Karasik *et al.* 2002d); a larger

Table 5 Gene × gene and gene × non-genetic factor interaction

Factors	Traits	Population	Effect	P value	Reference
1 ER- α × VDR	BMD	Pre- and perimenopausal women	(-/-)PvuII ER- α and bb VDR>(-/-) PvuII ER- α and BB VDR	<0.05	Willing <i>et al.</i> 1998
2 ER- α × VDR	Lumbar BMD	Italian postmenopausal women	AABBtt<aabbTT AABBtt-PPXX<aabbTT-ppxx	<0.05	Gennari <i>et al.</i> 1998
3 ER- α × VDR	Distal radius BMD changes	Postmenopausal women	For those with BB genotype, X and p associated with a larger increase (or smaller decrease) of drBMD, x and P associated with a smaller increase (or larger decrease)		Deng <i>et al.</i> 1998
4 VDR (FokI) × VDR (BsmI and ApaI)	LS BMD	Prepubertal whites	it was associated with a significant lower LS BMD in bb and aa individuals		Ferrari <i>et al.</i> 1998b
5 VDR BsmI × FokI	BMD	104 healthy men, aged 20.7–38.6 yr	Among BB, BMD were significantly lower in individuals carrying f allele		Ferrari <i>et al.</i> 1999
6 VDR × COL1A1	Fracture	1004 postmenopausal women	Fracture risk was dependent on the VDR 'baT' haplotype in only COL1A1 GT, TT group, not in COL1A1 GG group	0.03	Uitterlinden <i>et al.</i> 2001
7 ER- α × age	Weight	108 healthy postmenopausal white women	PP tend to gain weight with age, Pp and pp tend to lose weight with age		Deng <i>et al.</i> 2000b
8 VDR × age, dietary calcium intake	BMD	369 healthy white females, aged 7–56 yr	BMD associated with VDR gene polymorphism only before puberty (BB<other); BMD accrual was increased in Bb and possibly BB prepubertal girls after increasing dietary calcium intake, bb individuals had the greatest spontaneous BMD accrual and remained unaffected by calcium supplements		Ferrari <i>et al.</i> 1998a
9 VDR × race	Age- and BMI-adjusted BMD	83 white and 72 black women between 20 and 40 yr	No interaction of race by genotype on BMD		Fleet <i>et al.</i> 1995
10 Gene × HRT	Heel stiffness index	425 ambulatory postmenopausal French-Canadian women from Quebec aged 42–85 yr	Those with VDR-bb/ESR-PP genotype who received HRT for more than 5 yr had 21% greater heel stiffness index than those with the same genotype but who received HRT for less than 5 yr	0.002	Giguère <i>et al.</i> 2000
11 VDR × VitD supplementation	BMD increase	81 women, aged 70 yr and over	BMD increase in the VitD supplementation group was greater in the BB and Bb genotype, compared with bb genotype	0.03	Graafmans <i>et al.</i> 1997
12 Gene × race	BMD	154 premenopausal American women (72 black, 82 white)	No significant interaction between race and VDR FokI genotype		Harris <i>et al.</i> 1997
13 VDR BsmI × calcium intake	BMD	328 individuals aged 69–90 yr, 94 individuals aged 18–68 yr	In bb, not Bb or BB individuals, BMD was associated with calcium intake; BMD greater in persons with bb genotype only in the group with calcium intakes greater than 800 mg/day		Kiel <i>et al.</i> 1997
14 VDR × age	FN BMD	139 normal healthy women (53.2 ± 14.5 yr) and 43 severely osteoporotic postmenopausal women (65.8 ± 5.9 yr)	Age modulates the effect of VDR genotypes on FN BMD such that the effect of genotype was greatest among younger (premenopausal) women and declined with age so that there was no discernible difference by age 70 yr		Riggs <i>et al.</i> 1995
15 VDR × exercise VDR × calcium intake	BMD	677 healthy unrelated white women aged 18–35 yr	Genotype tt tend to have greater BMD than Tt and TT. This trend is most significant when daily calcium intake >684 mg		Rubin <i>et al.</i> 1999
16 VDR × weight	BMD	807 elderly women (>70 yr); 84 women with osteoporosis	Difference in FN BMD by BsmI genotype exists only in non-obese women (BMI <30 kg/m ²)		Vandevyver <i>et al.</i> 1997

Continued

Table 5 Continued

Factors	Traits	Population	Effect	P value	Reference
17 VDR × years since menopause	BMD	400 postmenopausal women of Italian origin	Association of FokI genotypes with lumbar BMD more significant among women in the first 5 yr of menopause	0.04	Gennari et al. 1999
18 VDR × caffeine	BMD	489 elderly women (aged 65–77), 96 controls	When caffeine intake >300 mg/day, spine bone loss of tt>TT	0.05	Rapuri et al. 2001
19 ER-α × HRT	BMD	322 early postmenopausal women	Long-term HRT eliminated ER-α genotype-related differences in BMD		Salmen et al. 2000
20 COL1A1 × age	BMD	1778 women, postmenopausal	BMD genotypic difference (SS>Ss>ss) increased with age		Uitterlinden et al. 1998
21 COL1A1 × dietary calcium intake	Bone loss	45 dizygotic twin pairs, 29 nuclear families with 120 individuals	LS bone loss, Ss and ss>SS for the lowest tertile of calcium intake; the opposite for the highest calcium intake	0.01, 0.03	Brown et al. 2001
22 VDR genotype × birthweight	BMD, BMC	165 men, 126 women aged 61–73 yr	Individuals in the lowest third of birthweight, spine BMD BB>other, spine BMC Ss and ss>SS	0.02	Dennison et al. 2001
23 ApoE*4 × estrogen status, HRT	Bone loss	392 healthy, pre-, peri-, and postmenopausal women	No difference in BMD and bone loss between different genotypes in premenopausal women. Bone loss of ApoE*4(+)>ApoE*4(–) in peri- and postmenopausal women. In peri- and postmenopausal women using HRT, no difference in rate of change in BMD between genotypes. Bone loss of ApoE*4(+)>ApoE*4(–) among non-HRT users.		Salamone et al. 2000
24 VDR × age	Bone loss	134 postmenopausal Irish women (age 58.19 ± 7.69 yr)	Older than 60 yr FF>Ff, ff Less than 60 yr: no association	0.03	Drummond et al. 2002
OPG × age			Older than 60 yr: CC>GC>GG Less than 60 yr: no association	0.01	
25 CYP19 × BMI	Baseline BMD, bone turnover markers, bone loss	300 elderly men	Difference in the studied traits between low and high TTTA repeat was greater in normal BMI individuals than when obese individuals were included		Gennari et al. 2002
CYP19 × testosterone			Increased CYP19 genotype effect in those with testosterone concentrations less than the median		
26 VDR (FokI, BsmI) × BMI	BMD	332 healthy early postmenopausal Danish women	BMI <25 kg/m ² : f allele associated with lower BMD of hip and forearm, b allele associated with lower spine BMD FF, BB women: no difference in BMD between obese and thin women		Tofteng et al. 2002

LS, lumbar spine; VitD, vitamin D; FN, femoral neck.

number of repeats tended to be associated with greater BMD in both studies. The TTTA repeat polymorphism of the *CYP19* gene was studied in both 1337 elderly women (Prince *et al.* 2002) and 300 elderly men (Gennari *et al.* 2002). The first study demonstrated that reduced hip BMD was associated with high TTTA repeat number (>12), but the second study revealed a greater rate of bone loss in individuals with low numbers of repeats (<9) than in those with high repeat rates (>9), implying potentially different effects of the polymorphism between the sexes. Relationships between α -2-HS glycoprotein gene polymorphism and bone-related phenotypes have been detected, namely for lumbar/femoral BMD (Dickson *et al.* 1994) and for calcaneal broadband ultrasound attenuation (Zmuda *et al.* 1998). More consistent results were achieved for the studies of apolipoprotein E gene polymorphism. Shiraki *et al.* (1997) reported the presence of the ApoE4 allele to be associated with lower lumbar spine BMD and higher bone turnover. Subsequently, Salamone *et al.* (2000) detected significantly greater annual bone loss in the individuals with the ApoE4 allele, providing evidence in support of the earlier findings.

Gene $\times$ gene and gene $\times$ non-genetic factor interactions

Gene $\times$ gene or gene $\times$ non-genetic factor interactions have been identified in many studies, reflecting multifactorial determination of bone mass and the complexities of the genetic basis underlying BMD (Table 5).

Gene $\times$ gene interaction involving the *VDR*, the *ER- α* , and the *COL1A1* genes was implied in a few studies. The genotype PP (*ER- α* PvuII) $\times$ bb (*VDR* BsmI) was associated with greater BMD values than PP $\times$ BB in white American women (Willing *et al.* 1998). In 1004 postmenopausal women (Uitterlinden *et al.* 2001), the fracture risk was found to be dependent on the baT haplotype of the *VDR* gene in only the *COL1A1* GT and TT, but not the GG genotype group. Another study in Italian women showed that the interaction could exist not only between different genes, but also among different loci of the same gene; the two haplotype groups, AABbTt and aabbTT, formed by the combination of the three polymorphism sites of the *VDR* gene (Gennari *et al.* 1998), differed significantly with respect to lumbar BMD. Such intra-gene/inter-locus interaction has also been identified in prepubertal white females (Ferrari *et al.* 1998b) and young adult males (Ferrari *et al.* 1999).

Non-genetic factors, including age (Riggs *et al.* 1995, Ferrari *et al.* 1998a, Uitterlinden *et al.* 1998, Drummond *et al.* 2002), race (Fleet *et al.* 1995, Harris *et al.* 1997), calcium intake (Kiel *et al.* 1997, Ferrari *et al.* 1998a, Rubin *et al.* 1999, Brown *et al.* 2001), vitamin D supplementation (Graafmans *et al.* 1997), HRT (Giguère *et al.* 2000, Salamone *et al.* 2000, Salmen *et al.* 2000), years since menopause (Gennari *et al.* 1999), caffeine intake (Rapuri *et al.* 2001), birthweight (Dennison *et al.* 2001), BMI

(Gennari *et al.* 2002, Tofteng *et al.* 2002), testosterone concentrations (Gennari *et al.* 2002) and exercise (Rubin *et al.* 1999) may also interact with genetic factors in determination of bone mass.

Because of its importance for gene–environment interaction in bone metabolism, the effect of calcium or vitamin D intake on the accrual or retention of BMD has been analyzed in several independent studies (Krall *et al.* 1995, Graafmans *et al.* 1997, Kiel *et al.* 1997, Ferrari *et al.* 1998a, Brown *et al.* 2001). The effect was manifested clearly only in those with the *VDR* gene Bb, and possibly the BB, genotypes, but not those with the bb genotype (Ferrari *et al.* 1998a). This finding is supportive of that by Graafmans *et al.* (1997), who also reported that an increase in BMD as a result of vitamin D supplementation was greater in BB and Bb individuals than in bb individuals. In contrast, the study by Kiel *et al.* (1997) showed that only the bb, rather the BB and Bb, individuals exhibited an increase in BMD in response to calcium intake. This inconsistency of genotype in individuals who benefit most from nutritional supplementation is probably attributable to their age differences, as participants in the study by Kiel *et al.* (1997) were in the age range 18–69 years, much older than the prepubertal participants in the study by Ferrari *et al.* (1998a). The effect of calcium intake in curbing postmenopausal bone loss may also vary for different sites. According to Krall *et al.* (1995), only the femoral neck bone loss in BB individuals could be alleviated significantly by calcium supplementation.

The above inconsistency underscores the importance of another factor, age, for its pivotal role in interacting with other factors in the determination of BMD. Riggs *et al.* (1995) studied 139 normal and 43 osteoporotic women, and found that the *VDR* genotypic difference with respect to BMD tended to blur with advancing age and eventually disappeared by the age of 70 years, implying erosion of genotypic differentiation of BMD by environmental effects accumulated over time. This is generally confirmed by the findings of another study in which BMD was associated with *VDR* polymorphisms only before puberty (Ferrari *et al.* 1998a), but is contradicted by those of a study in 1778 postmenopausal Dutch women, in whom BMD genotypic differentiation was accentuated by advancing age (Uitterlinden *et al.* 1998). A study by Drummond *et al.* (2002) demonstrated the complexity of the interplay between age and genetic factors. In that study, bone loss was found to be greater for the *VDR* FF and the osteoprotegerin gene (*OPG*) CC genotype than for the *VDR* Ff/ff and *OPG* GC/G genotype in individuals older than 60 years. As the *VDR* FF genotype was generally associated with greater BMD and the *OPG* C allele was associated with lower BMD (Wynne *et al.* 2002), it is to be expected that, with advancing age, BMD differentiation would tend to decrease in terms of *VDR* genotype and would tend to increase in terms of the *OPG* genotype. The overall effect of gene–age interaction on BMD of an

individual with both FF and CC genotype would thus depend on these two opposite trends of age-related change in BMD.

Gene–age interaction has also been suggested to influence weight, such that individuals with the *ER-α* PP genotype tended to gain weight and those with the Pp and pp genotypes lost weight with advancing age (Deng *et al.* 2000b). As weight and bone mass are highly correlated, the finding implies that the effect of gene–age interaction on BMD might be mediated through weight. In addition, weight – or probably height – itself may also potentially interact with the *VDR* polymorphism to influence BMD variation. Vandevyver *et al.* (1997) discovered, in a group of elderly women, that BMD differentiation among the BsmI genotypes could be detected only in non-obese women with BMI < 30 kg/m². Similarly, it was also reported that, among 332 postmenopausal Danish women, an association of the f and b alleles of the *VDR* gene with lower BMD could be found only in those with BMI less than 25 kg/m² (Tofteng *et al.* 2002). The findings are consistent with the conclusion of Deng *et al.* (1999) that covariates, such as weight and height, may obscure the associations of the *VDR* and *ER-α* genotypes with BMD.

HRT is another important environment factor that modulates genetic effects on bone. In a study by Deng *et al.* (1998), HRT tended to have differential effects for different *VDR* genotypes. In groups with the BB and Bb genotypes, HRT increased the distal radius BMD, but it tended to have the opposite effect on distal radius BMD in bb individuals. A study on 322 early postmenopausal women showed that differences in BMD that were related to the *ER-α* genotype were eliminated by long-term HRT (Salmen *et al.* 2000). HRT could also remove the difference in bone loss between the individuals with the ApoE4 allele and those without it (Salamone *et al.* 2000). Another study identified individuals with the *VDR* bb/*ER-α* PP genotype as a group who might benefit most from long-term HRT because, in that study, only those with the genotype bb-PP had an increased heel stiffness index in response to long-term HRT (Giguère *et al.* 2000).

Studies on race (white compared with African American) × *VDR* genotype interaction have not yielded positive effects (Fleet *et al.* 1995, Harris *et al.* 1997). This may be unexpected, given that the association between the *VDR* polymorphism and BMD is much more often detected in white Americans than in African Americans (Zmuda *et al.* 1997, 1999a).

Implications of monogenic bone diseases/traits and transgenic/knockout animal models for association studies

A series of candidate genes have been identified through the study of monogenic bone diseases/traits. Likewise, by means of knockout and transgenic animal models, many well-known or novel genes have been found to be

accountable for abnormal bone phenotypes. Studies on monogenic bone diseases and genetically manipulated animals can effectively complement association studies in elucidating the genetic factors of osteoporosis. They provide biologically relevant new candidate genes of osteoporosis for association studies to be tested in general populations. They can also help isolate and highlight the functions of a particular candidate gene, thus providing further evidence supporting the gene's relevance to variations in bone mass.

A salient example is the *TGF-β1* gene. Associated with lumbar spine BMD and markers of bone turnover in postmenopausal women, the gene has also been implicated in the pathogenesis of Camurati–Engelmann disease (Kinoshita *et al.* 2000), an autosomal dominant disease characterized by progressive hyperostosis and sclerosis of the diaphyses of the long bones. The importance of the gene to bone mass regulation was further validated in *TGF-β1*-knockout mice. Lack of the gene in these mice resulted in a significant decrease in bone mineral content and elasticity (Geiser *et al.* 1998). Recently, studies on three monogenic traits – autosomal recessive osteoporosis-pseudoglioma (Gong *et al.* 2001), autosomal dominant high bone mass (Johnson *et al.* 2002, Little *et al.* 2002), and autosomal recessive osteopetrosis (Heaney *et al.* 1998) – have brought to light another gene, the low-density lipoprotein receptor-related protein 5 gene (*LRP-5*) as a potential candidate gene for osteoporosis. The first two traits were proved to result from different mutations of the *LRP-5* gene (Gong *et al.* 2001, Little *et al.* 2002), and the last was linked to the genomic region, 11q12–13 (Heaney *et al.* 1998), which contains the gene. To confirm the significance of the gene for bone mass variation in the normal population, several independent studies were performed testing linkage of the genomic region, 11q12–13, with BMD. A sib-pair study with 374 sibships reported that a maximum logarithm of odds (LOD) score of 3.50 was achieved in this region for linkage to femoral neck BMD (Koller *et al.* 1998). However, a subsequent study by the same group with an expanded sample size of 595 sib pairs demonstrated a much reduced LOD score (<2.2) for linkage of this region to BMD (Koller *et al.* 2000). Moreover, the findings of a study by Deng *et al.* (2001a) did not suggest any significance of this region, and neither did several later large genome-wide linkage studies (Mitchell *et al.* 2001, Karasik *et al.* 2002a, Wilson *et al.* 2003).

For more detailed illustration of monogenic bone diseases and transgenic/knockout animal models for genetic studies of osteoporosis, the reader is referred to the reviews by Ralston (2002) and Peacock *et al.* (2002b).

Linkage studies

Linkage studies test whether a phenotypic locus is transmitted with genetic markers of known chromosomal

Table 6 Evidence for the presence of linkage with osteoporosis-related phenotypes

	Markers	Candidate genes	Phenotypes	P or LOD (Z) value	Reference
Location					
1p36	D1S450	<i>TNFRSF1B, PLOD</i>	Hip BMD	LOD=2.29	Devoto <i>et al.</i> 1998
	D1S214	<i>MTHFR</i>	Femoral neck BMD	LOD=3.53	Devoto <i>et al.</i> 2001
	D1S468		Quantitative ultrasound	LOD=2.74	Karasik <i>et al.</i> 2002b
			Whole-body BMD	LOD=2.4	Wilson <i>et al.</i> 2003
1q21-23	D1S484	<i>BGP, IL-6R</i>	Spine BMD	LOD=3.11	Koller <i>et al.</i> 2000
2p25			Spine bone size	LOD=1.54	Deng <i>et al.</i> 2002b
2p23-24	D2S149		Hip BMD	LOD=2.25	Devoto <i>et al.</i> 1998
2p21-24	D2S2976-D2S405	<i>CALM2, STK, POMC</i>	Proximal forearm BMD	LOD=2.15	Niu <i>et al.</i> 1999
			Distal forearm BMD	LOD=2.14	
2p21	D2S305	<i>COL6A3</i>	Spine bone size	LOD=2.15	Deng <i>et al.</i> 2002b
2q23	D2S160	<i>IL-1α</i>	Femoral neck BMD	LOD=1.4	Duncan <i>et al.</i> 1999
2q37	D2S125		Wrist bone size	LOD=2.28	Deng <i>et al.</i> 2002b
3p21	D3S1289, D3S3559	<i>PTHRI</i>	Femoral neck BMD	LOD=2.7 ~ 3.5	Duncan <i>et al.</i> 1999
			Spine BMD	LOD=2.1 ~ 2.7	Wilson <i>et al.</i> 2003
3p26	D3S1297		Wrist BMD	LOD=1.82	Deng <i>et al.</i> 2002c
3q12-26	D3S1271-D3S1614	<i>COL8A1, PLOD2</i>	Pelvis axis width	LOD=3.1	Koller <i>et al.</i> 2001
			Midfemur width	LOD=2.8	
			Femur head width	LOD=2.8	
4p16	D4S412	<i>FGFR3</i>	Wrist bone size	LOD=2.00	Deng <i>et al.</i> 2002b
			Hip BMD	LOD=2.2	
4p15	D4S2639	<i>BMP-3</i>	Radius midpoint BMD	LOD=4.05	Mitchell <i>et al.</i> 2001
4q11	D4S428		Femur neck axis length	LOD=3.9	Koller <i>et al.</i> 2001
			Midfemur width	LOD=3.5	
4q26	D4S429	<i>EGF</i>	Femoral neck BMD	LOD=1.8	Duncan <i>et al.</i> 1999
4q31			Spine BMD	LOD=3.08	Deng <i>et al.</i> 2002c
4q32	D4S413		Wrist BMD	LOD=2.26	Deng <i>et al.</i> 2002c
4q34	D4S1539		Hip BMD	LOD=2.95	Devoto <i>et al.</i> 1998
5p15.2	D5S817		Quantitative ultrasound	LOD=2.69	Karasik <i>et al.</i> 2002b
5p14	D5S2845		Trochanter BMD	LOD=1.75	Karasik <i>et al.</i> 2002a
5q12	D5S647-D5S644	<i>CRTL1</i>	Femur neck axis length	LOD=4.3	Koller <i>et al.</i> 2001
5q23	D5S2017	<i>IL-4</i>	Femoral neck BMD	LOD=1.2	Duncan <i>et al.</i> 1999
5q33-35	D5S422	<i>ON, PDGFRB</i>	Femoral neck BMD	LOD=1.87	Koller <i>et al.</i> 2000
6p21	D6S2427	<i>TNF-α</i>	Femoral neck BMD	LOD=2.93	Karasik <i>et al.</i> 2002a
			Lumbar spine BMD	LOD=1.88	
			Osteoporosis and osteopenia defined by radial bone BMD	P=0.001	Ota <i>et al.</i> 2000
6p11-12	D6S462	<i>BMP-6</i>	Spine BMD	LOD=1.94	Koller <i>et al.</i> 2000
6q25	D6S1577	<i>ER-α</i>	Lumbar spine BMD	LOD=1.4	Duncan <i>et al.</i> 1999
7p22	D7S531		Spine BMD	LOD=1.93	Deng <i>et al.</i> 2002c
			Spine BMD	LOD=1.62	Huang <i>et al.</i> 2002
7p21	D7S503	<i>IL-6</i>	Lumbar spine BMD	LOD=1.2	Duncan <i>et al.</i> 1999
8q24.3	D8S373	<i>TNFRSF11B</i>	Ward's BMD	LOD=2.13	Karasik <i>et al.</i> 2002a
9p24			Wrist BMD	LOD=1.87	Deng <i>et al.</i> 2002a
9q11-12			Wrist bone size	LOD=2.23	Deng <i>et al.</i> 2002b
9q21	D9S175	<i>COL5A1</i>	Wrist bone size	LOD=1.56	Deng <i>et al.</i> 2002b
10q26	D10S1651	<i>FGFR2</i>	Hip BMD	LOD=2.29	Deng <i>et al.</i> 2002c
			Hip BMD	LOD=1.65	Huang <i>et al.</i> 2002
11p15	D11S4046	<i>IGF-II</i>	Spine bone size	LOD=2.78	Deng <i>et al.</i> 2002b
11q12-13	D11S987	<i>LRP5</i>	Spine BMD	LOD=5.74	Johnson <i>et al.</i> 1997
	D11S1313		Spine BMD	LOD=1.97	Koller <i>et al.</i> 2000
11q24	CD3D		Spine BMD	LOD=2.08	Devoto <i>et al.</i> 1998
12q13	D12S83	<i>VDR, COL2A1</i>	Lumbar spine BMD	LOD=1.7	Duncan <i>et al.</i> 1999
	D12S368		HipBMD	LOD=1.69	Deng <i>et al.</i> 2002c
12q23			Lumbar spine BMD	LOD=2.08	Karasik <i>et al.</i> 2002a
12q24.2	D12S1723	<i>IGF-I</i>	Lumbar spine BMD	LOD=2.08	Deng <i>et al.</i> 2002c
13q21	D13S800		Hip BMD	LOD=3.1	Mitchell <i>et al.</i> 2001
13q34		<i>COL4A1, COL4A2</i>	Distal forearm BMD	LOD=1.67	Niu <i>et al.</i> 1999
	D13S285		Spine BMD	LOD=1.77	Deng <i>et al.</i> 2002c
			Spine BMD	LOD>1	Huang <i>et al.</i> 2002

Continued

Table 6 Continued

	Markers	Candidate genes	Phenotypes	P or LOD (Z) value	Reference
Location					
14q11			Hip bone size	LOD=1·65	Deng <i>et al.</i> 2002b
14q21·3	D14S587	<i>BMP-4</i>	Lumbar spine BMD	LOD=1·92	Karasik <i>et al.</i> 2002a
14q31·32			Trochanter BMD	LOD=1·99	Koller <i>et al.</i> 2000
15p11	D15S165		Spine BMD	LOD=1·6	Deng <i>et al.</i> 2002c
15q (56·62 cM)			Hip BMD	LOD=4·2	Peacock <i>et al.</i> 2002a
16q11·1-13	D16S753-D16S771		Serum osteocalcin	LOD=3·35	Mitchell <i>et al.</i> 2000
17p13	D17S1852		Wrist BMD	LOD=1·99	Deng <i>et al.</i> 2002c
17p11	D17S1857		Hip BMD	LOD=1·58	Deng <i>et al.</i> 2002c
17q21	D17S791	<i>COL1A1, CHAD, HOX</i>	Femur head width	LOD=3·6	Koller <i>et al.</i> 2001
17q23	D17S787	<i>TBX2</i>	Wrist bone size	LOD=3·98	Deng <i>et al.</i> 2002b
			Wrist bone size	LOD=1·18	Xu <i>et al.</i> 2002
	D17S807		Femoral neck BMD	LOD=1·7	Duncan <i>et al.</i> 1999
19p13	D19S226	<i>COMP, PRTN3</i>	Femur neck axis length	LOD=2·8	Koller <i>et al.</i> 2001
			Femur head width	LOD=2·8	
20p11·1-13·12	D20S447-D20S107	<i>CDMP1</i>	Serum osteocalcin	LOD=2·78	Mitchell <i>et al.</i> 2000
21q22-qter	D21S2055	<i>COL6A1, COL6A2</i>	Trochanter BMD	LOD=2·39	Karasik <i>et al.</i> 2002a
	D21S1446		Trochanter BMD	LOD=3·14	
22q12-13	D22S423		Spine BMD	LOD=2·13	Koller <i>et al.</i> 2000

BGP, osteocalcin; BMP-3, bone morphogenic protein-3; BMP-4, bone morphogenic protein-4; BMP-6, bone morphogenic protein-6; CALM2, calmodulin 2; CHAD, chondroadherin; CDMP1, cartilage-derived morphogenetic protein 1; COL1A1, type I collagen α 1; COL2A1, type II collagen α 1; COL4A1, type IV collagen α 1; COL4A2, type IV collagen α 2; COL5A1, collagen type V α 1; COL6A1, collagen type VI α 1; COL6A2, collagen type VI α 2; COL6A3, collagen type VI α 3; COL8A1, type VIII collagen α 1; COMP, cartilage oligomeric matrix protein; CRTLI, cartilage linking protein 1; EGF, epidermal growth factor; ER- α , estrogen receptor- α ; FGFR2, fibroblast growth factor 2; FGFR3, fibroblast growth factor 3; HOX, homeobox genes; IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IL-1 α , Interleukin-1 α ; IL-4, interleukin-4; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; LRP5, low density lipoprotein receptor-related protein 5; MTHFR, methylenetetrahydrofolate reductase; ON, osteonectin; PDGFRB, platelet-derived growth factor receptor- β ; PLOD, lysyl hydroxylase; POMC, pro-opiomelanocortin; PRTN3, proteinase 3; PTHR1, PTH receptor type 1; STK, serine/threonine kinase; TBX2, T-box 2; TNF- α , tumor necrosis factor α ; TNFRSF1B, tumor necrosis factor receptor subfamily 1B; TNFRSF11B, osteoprotegerin; VDR, vitamin D receptor.

position. The linkage approach does not rely on the linkage disequilibrium among genes or markers in adjacent genomic regions, and therefore it can be used to search for any genomic region that contributes relatively large variation in complex traits, without any prior knowledge. In contrast to the population association approach, the linkage approach is robust with respect to population admixture/stratification. However, genomic regions detected by linkage studies are generally large (~ 30 cM), and are thus not suitable for physical mapping; fine-mapping techniques are therefore needed to narrow down a QTL to a small region in order eventually to identify a specific gene (Deng & Chen 2000a, Deng *et al.* 2000a). The linkage approach has been successfully used to locate gene(s) underlying Mendelian inherited genetic traits, but its application to complex traits can be complicated (Lander & Schork 1994). In this case, it is necessary to screen or genotype (or both) very large samples (Risch & Merikangas 1996, Allison 1997, Xiong *et al.* 1998), or the power to detect linkage would be limited.

Linkage studies in humans

In recent years, the linkage approach has been applied to the search for genes associated with osteoporosis. Table 6 presents a summary of the loci showing evidence of linkage with BMD and other important risk determinants

of osteoporosis. These results are derived from either whole-genome linkage scans or candidate loci searches.

Whole-genome linkage scans Generally, genome-wide linkage scans are conducted by using panels of micro-satellite makers spaced uniformly throughout the entire human genome to identify QTLs affecting the traits. Sampling strategy involves gathering a large number of related individuals assumed to be segregated for genes that influence the traits. To date, genome-wide linkage scans are relatively rare in the field of bone genetics. Ten studies on osteoporosis-related phenotypes have been published. Among these, six focused on BMD (Devoto *et al.* 1998, Niu *et al.* 1999, Koller *et al.* 2000, Karasik *et al.* 2002a, Deng *et al.* 2002c, Wilson *et al.* 2003), two on bone size (Koller *et al.* 2001, Deng *et al.* 2002b), one on serum osteocalcin concentration (Mitchell *et al.* 2000) and one on quantitative ultrasound of the calcaneus bone (Karasik *et al.* 2002b). Abstracts for several other linkage analyses have also been published (Mitchell *et al.* 2001, Econs *et al.* 2002, Huang *et al.* 2002, Koller *et al.* 2002, Peacock *et al.* 2002a, Xu *et al.* 2002, Wilson *et al.* 2003), some with the purpose of confirming preliminary findings. General information on sampling strategies and molecular markers utilized in the eight full publications to date on whole-genome linkage studies are outlined and compared in Table 7. We also briefly review these studies according to study phenotype.

Table 7 Summary of whole genome linkage studies for osteoporosis-related phenotypes in humans

Study	Phenotypes	Sampling scheme	Study participants	Markers	Marker heterozygosity
Devoto et al. 1998	BMD at the spine and hip	Pedigrees ascertained by probands with low BMD	149 individuals from 30 nuclear pedigrees, containing 74 sib pairs	330 microsatellite markers	unknown
Niu et al. 1999	BMD at the proximal and distal forearm	Randomly ascertained Chinese nuclear families	218 individuals from 96 nuclear families, containing 153 sib pairs	367 microsatellite markers	0.77
Mitchell et al. 2000	Serum osteocalcin concentrations	A population-based family study, regardless of probands' medical history	429 individuals from 10 large multigenerational families	376 microsatellite markers	—0.74
Koller et al. 2000	BMD at the lumbar spine, trochanter, femoral neck and Ward's triangle	Randomly ascertained white and Africa-American healthy premenopausal women	595 sister pairs	270 microsatellite markers	0.70
Koller et al. 2001	Seven structural variables measured from femoral radiographs	Randomly ascertained healthy white women	481 sister pairs	270 microsatellite markers	0.70
Deng et al. 2002c	BMD at the spine, hip and wrist	Pedigrees ascertained by extreme probands with low BMD	635 individuals from 59 pedigrees, containing 11 391 informative relative pairs including 1249 sib pairs	380 microsatellite markers	0.79
Deng et al. 2002b	Bone size at the spine, hip and wrist	Pedigrees ascertained by extreme probands with low BMD	635 individuals from 59 pedigrees, containing 11 391 informative relative pairs including 1249 sib pairs	380 microsatellite markers	0.79
Karasik et al. 2002a	BMD at the femoral neck, trochanter, Ward's area and lumbar spine	Randomly ascertained extended families from the population-based Framingham Study	1164 individuals from 330 extended families	401 microsatellite markers	0.77
Karasik et al. 2002b	Quantitative ultrasound of the calcaneus bone	Randomly ascertained extended families from the population-based Framingham Study	1270 individuals from 324 extended families	401 microsatellite markers	0.77
Wilson et al. 2003	BMD at the lumbar spine, total hip and whole body	1) Unselected non-identical twin pairs 2) Highly selected, extremely discordant or concordant sib pairs	1) 2188 individuals from 1094 pedigrees 2) 587 individuals from 254 pedigrees	1) 737 microsatellite markers 2) A total of 1008 biallelic and microsatellite markers	Unknown

BMD The first genome-wide study for genomic loci involved in bone density was that by Devoto *et al.* (1998), in which a total of 149 individuals from seven large pedigrees (74 sib pairs) with low BMD were genotyped for 330 markers. The maximum LOD score obtained in parametric analyses was 2.08 for the marker CD3D at chromosome 11q for femoral neck BMD. Non-parametric analyses suggested linkage at chromosome 1p36 for femoral neck BMD ($Z_{\max}=3.51$ for D1S4450), 2p23-24 for spine BMD ($Z_{\max}=2.07$ for D2S149), and 4 qter for femoral neck BMD ($Z_{\max}=2.95$ for D4S1539 and $Z_{\max}=2.48$ for D4S1554). Niu *et al.* (1999) scanned the entire autosomal genome by genotyping 367 polymorphic markers for 218 individuals from 96 Chinese nuclear families (including 153 sib pairs). A genomic region covering 2p21.1-24 was linked to both proximal and distal forearm BMD (LOD=2.15 and 2.14 for proximal and distal forearm BMD respectively), and 13q34 was linked to distal forearm BMD with a multipoint LOD score of 1.67. Koller *et al.* (2000) performed a genome scan for BMD at the lumbar spine, trochanter, femoral neck and Ward's triangle using 429 healthy premenopausal white sister pairs. In their study, chromosomal regions showing LOD scores greater than 1.85 were further replicated in an expanded sample with 464 white and 131 African-American sister pairs. The highest LOD score of 3.86 was reached at chromosome 1q2-23 with lumbar spine BMD. Evidence of suggestive linkage was also found at 5q33-35 (LOD=2.23) and 11q12-13 (LOD=2.16) with femoral neck BMD, at 6p11-12 (LOD=2.13) with lumbar spine BMD.

Deng *et al.* (2002b) genotyped 380 microsatellite markers throughout the entire human genome for 635 white individuals from 59 extended pedigrees identified via probands with extremely low BMD values. This study contained more than 11 000 relative pairs (including 1249 sib pairs) informative for linkage analyses. For spine BMD, four putative genomic regions (4q31, 7p22, 12q24 and 13q33-34) were identified, with a greatest multipoint LOD score of 3.08 at 4q31. For hip BMD, the evidence of linkage was detected at the genomic regions 10q26, 12q13 and 17p11, with a maximum multipoint LOD score of 2.29 at 10q26. Suggestive linkage to wrist BMD was also found at genomic regions 3p26, 4q32, 9p22-24 and 17p13. Another genome-wide scan was performed in a randomly ascertained set of 330 healthy white pedigrees from the population-based Framingham Study (Karasik *et al.* 2002a). The investigators reported suggestive two-point linkage of BMD at the femoral neck to 6p21.2 (LOD=2.93), linkage of trochanteric BMD to 21q22.2 (LOD=2.34), and possible linkage of lumbar spine BMD to 12q24.2 and 6p21.2. In the multipoint analysis, strong evidence of linkage was shown with trochanteric BMD at 21 qter (D21S1446) (LOD=3.14). Other regions of possible linkage (LOD>2.2) were at 21q22.2 with trochanteric BMD, 8q24 with Ward's area, and 14q31

with lumbar spine BMD. Wilson *et al.* (2003) performed independent genome-wide screens on two complementary cohorts: unselected non-identical twin pairs (1094) and highly selected extremely discordant or concordant (EDAC) sib pairs (254 pedigrees). The evidence of linkage in the unselected twins (spine BMD, LOD=2.7) and the EDAC pedigrees (spine BMD, LOD=2.1) was observed at genomic region 3p21. Furthermore, suggestive linkage was found in twin cohorts for whole-body BMD (LOD=2.4) at 1p36.

In a study in 898 individuals from 34 Mexican-American families, reported at the preliminary stage (Mitchell *et al.* 2001), significant linkage was detected at genomic region 4p (LOD=4.05) to BMD at the midpoint radius. Additional evidence of linkage to BMD at the hip (intertrochanteric region) was also observed at the same region (LOD=2.2).

Bone size Koller *et al.* (2001) genotyped 270 markers for 309 white sister pairs to identify genomic regions underlying normal variation of femoral structure (including seven structure variables). Three chromosomal regions – 5q, 4q and 17q – were identified as having significant evidence of linkage (LOD>3.6) to at least one femoral structure phenotype. In another study, for a sample of 53 pedigrees that included 1249 sib pairs, Deng *et al.* (2002b) performed a whole-genome scan using 380 microsatellite markers to identify genomic regions potentially containing QTLs important for variation in bone size (measured by dual-energy X-ray absorptiometry). For variation in wrist (ultra distal) bone size, a LOD score of 3.89 was achieved at D17S787 in two-point linkage analyses and a LOD score of 3.01 was achieved at 17q23 in multipoint analyses. For variation in hip bone size, a LOD score of 1.99 was achieved at D19S226 in two-point analyses, and a LOD score of 2.83 was achieved at 19p13 in multipoint analyses.

Serum osteocalcin concentration Serum osteocalcin concentration has been regarded as a biochemical marker of bone turnover. A genome-wide scan of serum osteocalcin concentration in 429 individuals from 10 pedigrees (Mitchell *et al.* 2000) revealed significant linkage at genomic region 16q11.1-13 (LOD=3.35), and suggestive linkage on 20q11.1-13.12 (LOD=2.78).

Quantitative ultrasound Karasik *et al.* (2002b) conducted a whole-genome scan in 324 white families (1270 measured individuals) from the Framingham Osteoporosis Study to identify genomic regions that may harbor genes influencing variation in calcaneal ultrasound measures (including broadband ultrasound attenuation, speed of sound and quantitative ultrasound index). Evidence of linkage was detected on chromosomes 1p36.3 and 5p15.2.

Linkage studies on candidate loci Some linkage studies were performed in candidate genomic loci or

targeted chromosomal regions. In a sib-pair linkage study of 23 candidate genes, Duncan *et al.* (1999) genotyped 64 microsatellite markers in a sample of 115 probands identified from patients with primary osteoporosis and 499 of their relatives. Suggestive linkage was found between BMD and the *PTHR1* gene (LOD>2.7). Suggestive evidence of linkage was also observed with the genes for epidermal growth factor, *COLIA1*, *COLIA2*, *VDR*, *ER- α* , *IL- α* , *IL-4* and *IL-6*. In another study, Ota *et al.* (1999) tested 192 sibling pairs of adult Japanese women from 136 families for genetic linkage between osteopenia and allelic variants of four candidate genes (*IL-6*, *IL-6R*, *CaSR*, and *MGP*). Significant linkage of the *IL-6* locus to osteopenia was observed by qualitative methods. Evidence of linkage was also found between low BMD and the *IL-6* locus by quantitative methods. In the same study sample, Ota *et al.* (2000) observed linkage between osteoporosis and osteopenia and allelic variants at the tumor necrosis factor- α (*TNF- α*) locus. In a sample of 542 healthy premenopausal sibling pairs (418 white and 124 African-American pairs), Takacs *et al.* (1999) did not find evidence of linkage between BMD and the *IGF-I* gene polymorphism for spine or hip BMD. In a similar study, Takacs *et al.* (2000) found no evidence for either linkage or association between the *IL-6* gene locus and BMD at the spine or femoral neck.

Deng *et al.* (2002a) simultaneously tested linkage, association, or both (via the transmission disequilibrium test) for three candidate genes (*VDR*, *BGP* and *PTH*) as putative QTLs underlying spine or hip BMD variation. Their data support the *VDR* gene as a QTL underlying spine BMD variation and *BGP* as a QTL underlying hip or spine BMD variation. Using similar statistical methods, Andrew *et al.* (2002) tested linkage and association for BMD and heel ultrasound measurements near the osteocalcin gene in female dizygotic twins. Strong evidence for pleiotropic linkage was found for broadband ultrasound attenuation and BMD in postmenopausal women.

Human chromosomal region 11q12-13 has been of great interest for many investigators in the bone genetics field during the past few years. Three distinct Mendelian inherited traits that are BMD related, including osteoporosis-pseudoglioma (Gong *et al.* 1996), autosomal recessive osteopetrosis (Heaney *et al.* 1997), and an autosomal dominant trait characterized by high bone mass (*HBM*) (Johnson *et al.* 1997), have been linked to this region. Little *et al.* (2002) refined this region with the expanded *HBM* pedigrees. A systematic search for mutations that segregated with the *HBM* phenotype revealed that a mutation in the *LRP-5* gene results in the *HBM* phenotype. Subsequent studies demonstrated that the *LRP5* V171 mutation causes high bone density, with a thickened mandible and torus palatinus, by impairing the action of a normal antagonist of the wingless type (Wnt) pathway and thus increasing Wnt signaling (Boyden *et al.* 2002). A study in 374 sib pairs for seven microsatellite

markers typed in 11q12-13 (Koller *et al.* 1998) reported linkage to normal femoral neck BMD variation (LOD=3.50 with marker D11S987). However, a subsequent study (Koller *et al.* 2000) with an expanded sample size (595 sib pairs) revealed much reduced signal for linkage, with a LOD score less than 2.2 achieved at D11S987. Deng *et al.* (2001b) genotyped five markers in this region (~27 cM centering on D11S987) for 635 individuals from 53 extended pedigrees (including 1249 sib pairs), but did not find linkage involving BMD at the spine, hip, wrist or total body BMD.

Replications Several genomic regions have been replicated across different studies. The genomic region 2p23-24 linked to spine BMD in white populations (Devoto *et al.* 1998) overlaps the region 2p21.1-24 revealed by Niu *et al.* (1999) for both proximal and distal forearm BMD in Chinese sib pairs. A number of candidate genes, such as calmodulin 2 (*CALM2*), serine/threonine kinase (*STK*) and pro-opiomelanocortin (*POMC*) reside in this region. A genome scan of 1097 female UK twin pairs (Wilson *et al.* 2003) confirmed the presence of QTLs for BMD at 1p36 and 3p21 highlighted by Niu *et al.* (1999) and Duncan *et al.* (1999). Important candidate genes, including *TNFR2* and *PTHR1*, are located at 1p36 and 3p21 respectively. There is also potential evidence for replication on chromosomal region 4q31 and 13q34 in three different studies (Duncan *et al.* 1999, Niu *et al.* 1999, Deng *et al.* 2002c). Two candidate genes (*COL4A1* and *COL4A2*) are located at 13q34.

Several groups have made extensive efforts in either independent or expanded samples (partially overlapping with initial samples) to confirm the findings in their initial stage studies. To confirm the QTLs influencing BMD revealed by Deng *et al.* (2002c), Huang *et al.* (2002) conducted a second-stage linkage study with more dense markers within the initial localized regions in expanded pedigrees. Evidence of potential replication (LOD>1.0) was found at several regions, such as 10q26, 7p22, 12q13 and 13q33-34. A study by the same group (Xu *et al.* 2002) also replicated a QTL at 17q23 that influenced the variation in wrist bone size revealed by Deng *et al.* (2002b). Another group reported replications of their initial-stage results on chromosome 1q for spine BMD (Econs *et al.* 2002), on chromosomes 14 and 15 for hip BMD (Peacock *et al.* 2002a), and at chromosomes 3 and 9 for femoral structure (Koller *et al.* 2002).

Statistical power of current linkage studies for osteoporosis

The disagreement in results between different linkage studies may reflect the complexity of genetic inheritance of osteoporosis. Other possible reasons may lie in the diversity of study designs, sample sizes, ascertainment schemes and statistical analyses used. From the statistical genetics point of view, power issues involving linkage

analysis are of critical importance in interpreting the inconsistency across the different studies.

Currently, two basic analytical approaches of model-free linkage analysis – the sib-pair method (Haseman & Elston 1972) and variance components method (Amos 1994, Almasy & Blangero 1998) – are routinely used in osteoporosis gene mapping. The merit of the sib-pair method is its relative simplicity of design and computation, but it is of limited power when the sample size is small or when sibling pairs are ascertained randomly (Risch & Merikangas 1996, Xiong *et al.* 1998). For instance, more than 8000 randomly ascertained sibling pairs are needed in a whole-genome scan to detect a major locus with a heritability as large as 30% (Xiong *et al.* 1998). The variance components method may provide a more powerful test, which can be expanded to deal with pedigrees of arbitrary size and complexity (Williams & Blangero 1999); generally, the larger and more complex the sample, the more powerful is this method (Williams & Blangero 1999). Several other factors, such as marker heterozygosity, genotyping error rate and marker density, may also affect the power to detect a QTL. In addition, sampling through extreme probands may improve the linkage power (Risch & Zhang 1995, Deng & Li 2002).

We discuss here the potential problems of the inference made in several earlier studies, with the hope of interpreting the linkage findings correctly. In a recent genome-wide scan study in 595 white and black sister pairs, Koller *et al.* (2000) reported several genomic regions that may harbor QTLs affecting BMD; a high LOD score (3.86) was achieved. In the two subsequent extension studies with fewer than 800 sib pairs, the same group replicated several of their major linkage findings (Econs *et al.* 2002, Peacock *et al.* 2002a). We calculated the statistical power for 595 independent sister pairs (Koller *et al.* 2000) and compared it with data from another study (Deng *et al.* 2002c) in which 53 pedigrees containing 1249 sibling pairs were analyzed. Koller *et al.* (2002) did not provide the overall heritability (h^2) of BMD in their sample; therefore, we set the overall heritability (h^2) of BMD as 0.60 (Peacock *et al.* 2002b). We assumed the frequency of the allele causing a decrease in BMD to be 0.40, a relatively liberal situation for achieving high statistical power. The power for sib pairs is computed using the program Genetic Power Calculator (Sham *et al.* 2000) (available at <http://statgen.iop.kcl.ac.uk/gpc/>). The power of the complex pedigrees (Deng *et al.* 2002c) was estimated via simulations by using the program simqtl in SOLAR (Blangero & Almasy 1997) (available at <http://www.sfbr.org/sfbr/public/software/solar/>). The results showed that, even when the QTL effect was high (h^2 attributable to the QTL is 0.25), and the marker was the QTL ($\vartheta=0$), the power of the study (Koller *et al.* 2000) was less than 30% (Fig. 1). The power decreased dramatically when the distance of marker and the QTL was ~ 5 cM ($\vartheta=0.05$). Compared with sib pairs (Koller *et al.* 2000), the complex pedigrees

(Deng *et al.* 2002c) had much higher power; nevertheless, it was still insufficient to detect QTLs powerfully when h^2 was less than 30%.

Because of polygenic inheritance, in a whole-genome scan some QTLs may be detected even if the statistical power is low (Deng *et al.* 2001b). However, to detect linkage of a specific QTL (e.g. to replicate a previous linkage finding), the statistical power required under a high LOD score should be sufficient. We also calculated the sample sizes required (with 80% statistical power) to detect QTLs at a LOD score of 3.0 with sib pairs. The number of sib pairs required is rather large (from 945 to 10 580) when the QTL effect is intermediate (h^2 attributable to the QTL is between 0.10 and 0.30) (Table 8). The empiric ability to replicate previous whole-genome linkage results (Econs *et al.* 2002, Peacock *et al.* 2002a) with fewer than 800 sib pairs seems to suggest the existence of a major gene with a h^2 greater than 30%. Only, and even if, with such a dramatic magnitude of h^2 attributable to a single QTL, can we have 80% power to detect it with at least 945 sib pairs. With the existence of such a major gene in humans, some other studies (e.g. Deng *et al.* 2002c, Karasik *et al.* 2002a, Wilson *et al.* 2003) might have had a greater power to detect it in similar populations; however, this has not been the case.

The above power analyses suggest that: (1) a high LOD score does not necessarily indicate that the study is more powerful or that the results are more robust than those of other studies; (2) it is inappropriate to use the negative findings of linkage studies that have insufficient power as a gold standard to judge the importance of candidate genes, as these genes may have relatively small or moderate effects on the studied traits; (3) without sufficient statistical power, it will be a challenge to resolve the continuously appearing significant (within individual groups) but yet largely inconsistent (across study groups) results from linkage analyses.

QTL mapping in animals

QTL mapping in animal models provides a complementary tool to identify genes involved in complex human diseases, with a key assumption that major regulatory genes will be shared among different species. The mouse models have been most often used, with a preliminary study also reported in the baboon.

Starting with two completely inbred parental lines, a number of line-cross populations derived from the F1 can be used for QTL mapping. In BMD-related mouse models, the basic idea is to set up experimental crosses of mouse strains with low BMD and high BMD, and then backcross the F1 to one parental line to generate the backcross lines, or to intercross the F1 population to generate the F2 mice with markedly varying BMD. Besides the backcross lines and the F2 mice, other

Figure 1 Comparison of the statistical power of 595 independent sib pairs (Koller *et al.* 2000) (dotted lines) and pedigrees composed of 630 individuals from the data of Deng *et al.* (2002c) (solid lines). (A) Recombination fraction = 0. (B) Recombination fraction = 0.05. ---◆--- additive, 595; ---■--- dominant, 595; ---▲--- recessive, 595; ---×--- additive, 630; ---△--- dominant, 630; ---*--- recessive, 630.

line-cross populations may provide an alternative strategy. For instance, recombinant inbred lines, which can be constructed by taking an F1 line through several rounds of selfing or brother-sister mating, may allow marker-trait associations to be scored in a completely homozygous background and across several environments (Lynch & Walsh 1998). QTL mapping then can be conducted in

experimental crosses with sufficient polymorphic molecular markers spanning the whole genome or candidate loci. Once the chromosomal regions harboring murine QTLs have been identified, the homologous human chromosomal regions may be revealed by human-mouse conserved synteny maps. We summarize the current findings of QTLs important in mice and the corresponding human

Table 8 Sample sizes required to achieve 80% power with a LOD score of 3.0

	Additive		Dominant		Recessive	
	$\theta=0.00$	$\theta=0.05$	$\theta=0.00$	$\theta=0.05$	$\theta=0.00$	$\theta=0.05$
h^2_q						
0.05	22 380	34 130	23 290	36 820	24 610	38 390
0.10	6179	9437	6442	10 260	6800	10 580
0.15	3007	4602	3141	5036	3311	5178
0.20	1838	2819	1922	3103	2024	3182
0.25	1269	1952	1329	2159	1398	2209
0.30	945	1458	992	1619	1042	1654
0.35	741	1145	778	1277	817	1302
0.40	602	933	632	1043	664	1062

h^2_q is the heritability due to the QTL under study; θ is the recombination fraction between the QTL and a marker.

homologous regions in Table 9. The study strategies of the available whole-genome QTL mapping for BMD-related traits in mice are reviewed in Table 10.

Using 24 recombinant inbred BXD strains, derived from a cross between progenitors of C57BL/6 (with a low BMD) and DBA/2 (with a high BMD), Klein *et al.* (1998) detected 10 chromosomal regions linked to peak BMD development in female mice. In the subsequent study with three additional independent populations derived from the same progenitors, Klein *et al.* (2001) confirmed their findings on chromosomes 1, 2, 4 and 11. Shimizu *et al.* (1999) reported a whole-genome scan for QTLs underlying peak BMD variation with the F2 intercrosses of SAMP6 (with a low BMD) and SAMP2 (with a high BMD). Significant evidence of linkage was identified on chromosomes 11 (LOD=10.8) and 13 (LOD=5.8). In a further study, Shimizu *et al.* (2001) elucidated the effects of the chromosome 13 locus (*Pbd2*) on peak BMD using SAM strains and a congenic strain, P6.P2-Pbd2^b. Their data confirmed the existence of a *Pbd2* locus that regulates peak BMD in the SAM strains. Benes *et al.* (2000) analyzed crosses between SAM6 (with a low BMD) and either AKR/J or SAM1 (with a high BMD). They detected multiple QTLs regulating spine BMD on chromosomes 2, 7, 11, 13 and 16, some of which coincide with those identified in a previous study (Klein *et al.* 1998).

Beamer *et al.* (1999) performed a genome scan in a cohort of F2 mice derived from intercross mating of (C57BL/6J × CAST/EiJ) F1 parents and identified four QTLs (Bmd1, Bmd2, Bmd3 and Bmd4). In another study, Beamer *et al.* (2001) analyzed 986 B6C3 F2 female inbred strains derived from C57BL/6J (B6) and C3H/HeJ (C3) mice. A total of 10 chromosomes (1, 2, 4, 6, 11, 12, 13, 14, 16 and 18) carrying QTLs for femur BMD and seven chromosomes (1, 4, 7, 9, 11, 14 and 18) carrying QTLs for vertebral BMD were found. Drake *et al.* (2001b) investigated the phenotypic and genetic relationships among BMD-related traits and those of adipose tissue and plasma lipids in mice with diet-induced atherosclerosis. Six loci that linked to bone-related traits on chromosomes 2, 3, 6,

7 and 15 were identified, three of which coincided with the loci linked to adipose tissue and plasma high-density lipoprotein. In another study, Drake *et al.* (2001a) conducted a genome screen with 16-month-old female F2 progeny of a C57BL/6J × DBA/2J intercross for measures of femur length and width. Evidence of linkage was found on proximal chromosome 3 (LOD=6.1) and chromosome 14 for femur length. A QTL affecting width of the diaphysis was found on chromosome 7 (LOD=6.8). Suggestive linkage for width at the intertrochanteric region was found on chromosomes 6 and 19. Zhang *et al.* (2002) presented a preliminary report of a locus on chromosome 4 (LOD=18.4) that influenced BMD in BXD mice (C57BL/6 × DBA/2 recombinant strains), in addition to a locus on chromosome 19 (LOD=13.3) that influenced osteoclastogenesis. The only available study using the baboon model revealed a chromosomal region (homologous to human 11q12) linked to BMD variation (Mahaney *et al.* 1997).

Summary

Understanding of osteoporosis has evolved within the past decade, thanks to extensive molecular genetics research. With the important findings available as summarized in this article, the genetic basis of osteoporosis becomes more finely delineated, as witnessed by the identification of a list of candidate genes, genomic regions and interactions between genes and environment that underlie variation in bone-related traits. However, the majority of these findings perhaps remain inconclusive pending further investigation, given that a large number of conflicting or contradictory discoveries persist for which the reasons are unidentified. Reflecting the complicated inheritance patterns of osteoporosis as a complex disease, the inconsistencies call for new approaches and strategies that have both sensitivity and robustness to accommodate confounding effects from various sources, including genetic heterogeneity, population admixture and gene-gene or gene-environment interactions, to name but a few. Only on the

Table 9 Quantitative trait loci (QTLs) reported for animal models with their putative homologous human location

	MGD map position (cM)	LOD or <i>P</i> value	Phenotype	Chromosome		Reference
				Mouse	Human	
Markers of gene						
cfh	74.1	<i>P</i> =0.0093	Whole-body BMD	1	1q25;1q31-41	Klein <i>et al.</i> 1998
D1Mit14	81.6	LOD=24.4	Femur BMD		1q23-31	Beamer <i>et al.</i> 2001
		LOD=14	Vertebral BMD			
D1Mit15	87.9	<i>P</i> <<0.01	Femur BMD		1q21-25	Beamer <i>et al.</i> 1999
D2Mit312	1	<i>P</i> =0.003	Spine BMD	2	10p15	Benes <i>et al.</i> 2000
D2Mit119	5	<i>P</i> <0.0001	Spine BMD		10p13-15	Benes <i>et al.</i> 2000
Il2ra	6.4	<i>P</i> =0.0071	Whole-body BMD		10p11-15	Klein <i>et al.</i> 1998
D2Mit464	9.5	<i>P</i> <0.0001	Spine BMD		10p11-15;2q14;9q34	Benes <i>et al.</i> 2000
D2Mit296	18	<i>P</i> <0.0001	Spine BMD		9q33-34	Benes <i>et al.</i> 2000
D2Mit413	84.2	LOD=3.8–3.9	Femur BMD		20p11-q12	Drake <i>et al.</i> 2001 <i>b</i>
D2Mit63	65.2	LOD=4.59	Femur BMD		15q15-21	Drake <i>et al.</i> 2001 <i>a</i>
lpls2-4	86	<i>P</i> =0.002	Whole-body BMD		20p11-q12	Klein <i>et al.</i> 1998
D2Mit456	86.3	LOD=3.14	Femur BMD		20p11-q12	Beamer <i>et al.</i> 2001
D3Mit14	64.1	LOD=2.5;3.3	Femur BMD	3	1p13;4q24-27	Drake <i>et al.</i> 2001 <i>b</i>
D3Mit51	35.2	LOD=6.09	Femur BMD		3q25	Drake <i>et al.</i> 2001 <i>a</i>
D4Mit124	57.4	LOD=16.3	Femur BMD	4	1p31-35	Beamer <i>et al.</i> 2001
		LOD=14.85	Vertebral BMD			
D4Mit186	44.6	LOD=18.4	Femur BMD		1p32-31	Zhang <i>et al.</i> 2002
D4Mit54	66	LOD=4.2	Femur BMD		1p34-36	Drake <i>et al.</i> 2001 <i>a</i>
D5Mit112	42	<i>P</i> =0.01	Femur BMD	5	4p12-14;4q11-13;4q21	Beamer <i>et al.</i> 1999
D6Mit150	51	LOD=4.56	Femur BMD	6	3p25-26;3q21-24;19q13;	Beamer <i>et al.</i> 2001
					10q11	
D6Mit198	67	LOD=2.3;2.7	Femur BMD		12p12;12q23-24	Drake <i>et al.</i> 2001 <i>b</i>
D6Mit198	67	LOD=4.35	Femur BMD		12p12;12q23-24	Drake <i>et al.</i> 2001 <i>a</i>
D7Mit210	11	<i>P</i> <0.001	Spine BMD	7	19q12-13	Benes <i>et al.</i> 2000
D7Mit227	16	<i>P</i> =0.001	Spine BMD		19q12-13	Benes <i>et al.</i> 2000
D7Mit80	18	LOD=2.04;2.37	Femur BMD		19q12-13	Drake <i>et al.</i> 2001 <i>b</i>
D7Mit7	1.7-65.2	LOD=6.85	Femur BMD		7q32;11q15;11q11-14;	Drake <i>et al.</i> 2001 <i>a</i>
					15q11-26;16p13;19q13	
D7Mit234	44	<i>P</i> =0.0007	Whole-body BMD		6p24-25;15q23-25;11q13-22;	Klein <i>et al.</i> 1998
					11p15	
D7Mit332	65.6	LOD=5.01	Vertebral BMD		10q24-26	Beamer <i>et al.</i> 2001
D9Mit196	48	LOD=5.12	Vertebral BMD	9	6q12-16;15q24-25;3q21-24	Beamer <i>et al.</i> 2001
D11Mit242	31	LOD=6.76	Femur BMD	11	5q31-32;17p11-12	Beamer <i>et al.</i> 2001
		LOD=2.98	Vertebral BMD			
D11Mit59,90	42-58.5	LOD=10.8	Femur BMD		17p11-13;17q11-13;17q21-23;	Shimizu <i>et al.</i> 1999
					1p36	
D11Mit284	52	<i>P</i> <0.0001	Spine BMD		17q21-23	Benes <i>et al.</i> 2000
D11Mit14	57	<i>P</i> =0.0104	Whole-body BMD		17q11-12;17q21-23	Klein <i>et al.</i> 1998
D11Mit160	58	<i>P</i> <0.0001	Spine BMD		3p21;17q11-12;17q21-23	Benes <i>et al.</i> 2000
D12Mit215	2	LOD=2.89	Femur BMD	12	2p22-25	Beamer <i>et al.</i> 2001
D13Mit174,135	9–10	LOD=5.8	Femur BMD	13	7p13-15;6p21-23;9q22	Shimizu <i>et al.</i> 1999
D13Mit16	10	<i>P</i> <0.1	Femur BMD		7p13-15;6p22;9q22	Beamer <i>et al.</i> 1999
D13Mit13	35	LOD=7.73	Femur BMD		5q22-32;9q22	Beamer <i>et al.</i> 2001
D13Mit20	35	<i>P</i> =0.001	Spine BMD		5q22-32;9q22	Benes <i>et al.</i> 2000
Ptprg	2	<i>P</i> =0.0007	Whole-body BMD	14	3p14;3p24;10q21-24;8p23	Klein <i>et al.</i> 1998
D14Mit160	40	LOD=4.3	Femur BMD		8p21-22;13q14-21	Beamer <i>et al.</i> 2001
		LOD=4.48	Vertebral BMD			
D14Mit35	44	LOD=3.43	Femur BMD		13q14-21	Drake <i>et al.</i> 2001 <i>a</i>
D15Mit13	6.7	LOD=2.97;3.21	Femur BMD	15	5p12-14;5q31	Drake <i>et al.</i> 2001 <i>b</i>
D15Mit206	17.2	LOD=3.19-4.73	Femur BMD		8q22-23	Drake <i>et al.</i> 2001 <i>b</i>
D15Mit29	42.8	<i>P</i> <0.01	Femur BMD		8q21;8q24;22q12-13	Beamer <i>et al.</i> 1999
Atf4	43.3	<i>P</i> =0.0099	Whole-body BMD		8q21;8q24;22q12-14	Klein <i>et al.</i> 1998
D16Mit100	8.5	<i>P</i> =0.02	Spine BMD	16	8q11;22q11	Benes <i>et al.</i> 2000
Hmg1-rs7	19	<i>P</i> =0.0055	Whole-body BMD		3q13;3q21-22;3q25;3q27-29	Klein <i>et al.</i> 1998
D16Mit12	27.6	LOD=4.07	Femur BMD		3q13-21;3q25;3q29	Beamer <i>et al.</i> 2001
D16Mit39	29.1	<i>P</i> =0.001	Spine BMD		3q13-21;3q28-29	Benes <i>et al.</i> 2000

Continued

Table 9 Continued

	MGD map position (cM)	LOD or <i>P</i> value	Phenotype	Chromosome		Reference
				Mouse	Human	
Markers of gene						
D18Mit36	24	LOD=13.67 LOD=8.35	Femur BMD Vertebral BMD	18	5q21-33	Beamer <i>et al.</i> 2001
D18Ncvs23	48	<i>P</i> =0.0094	Whole-body BMD		18q21	Klein <i>et al.</i> 1998
Nds1	43	LOD=13.3	Osteoclastogenesis	19	10q22-24	Zhang <i>et al.</i> 2002
D19Mit8	16-53	LOD=3.15	Femur BMD		9q12-21;9p24;10q21-25	Drake <i>et al.</i> 2001a
D19Ncvs21	53	<i>P</i> =0.0093	Whole-body BMD		10q24-26	Klein <i>et al.</i> 1998

MGD, Mouse Genome Database available at <http://www.informatics.jax.org>. Human homologous regions correspond to ± 3 cM of published marker or gene location identified at the MDG web site.

Table 10 Summary of the whole genome QTL mapping for BMD-related traits in mice

	Parents	Population	Sample size	Method	Markers	Traits
Reference						
Klein <i>et al.</i> 1998	C57BL/6 DBA/2	RI	24	Single marker analysis	1522	Whole-body BMD
Shimizu <i>et al.</i> 1999	SAMP6 SAMP2	F2	246	Interval mapping approach Multiple regression	90	Cortical thickness index
Beamer <i>et al.</i> 1999	C57BL/6J CAST/Eij	F2	714	Marker-based regression	127	Femoral BMD
Benes <i>et al.</i> 2000	SAMP6 AKR/J; SAMR1	F2	250	Univariate linear regression Stepwise multivariate regression Composite interval mapping	227	Spinal BMD
Drake <i>et al.</i> 2001b	C57BL/6J DBA/2J	F2	141	Interval mapping Composite interval mapping	Unknown	Femoral BMD
Drake <i>et al.</i> 2001a	C57BL/6J DBA/2J	F2	141	Interval mapping	Unknown	Femoral bone length and width
Beamer <i>et al.</i> 2001	C57BL/6J C3H/HeJ	F2	986	Genome-wide analyses Multiple regression	107	Femoral BMD Vertebral BMD

basis of such sound methodologies can the controversies be resolved and real breakthroughs be made in the search for osteoporosis genes.

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