OSTEOPOROSIS: GENETIC ANALYSIS OF MULTIFACTORIAL DISEASE

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Osteoporosis is a common disorder with a complex pathophysiology involving both endogenous and environmental factors. Family and twin studies have shown that genetic factors play an essential role in bone mass regulation and that apart from rare instances the heritability of bone mineral density (BMD) and osteoporosis is polygenic. Linkage analysis and association studies with numerous DNA markers (single nucleotide polymorphisms or microsatellites) have analysed several bone-related candidate genes encoding vitamin D, calcium-sensing, calcitonin and estrogen alpha receptors, insulin growth factor I, collagen type I alpha 1 chain and others. Despite this, the definite polymorphic marker has not been found in different populations which reflects the divergent results of association studies with their frequent limitations, and probably the fact that the relevant polymorphism is still awaiting identification. Once the genetic determinants can be defined, the clinical implications would be extensive both in diagnostics and in pharmacogenetics.

Key words: Osteoporosis – Bone mineral density – Candidate genes – DNA polymorphisms – Association studies

Osteoporosis is a systemic skeletal disease characterized by low bone mass and the microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fractures (CONSENSUS 1993). The disease is defined to exist when bone mineral density (BMD) values as estimated by dual energy X-ray absorptiometry (DXA) fall for more than 2.5 standard deviations below the young adult mean (KANIS et al. 1994).

The pathophysiology of osteoporosis is complex, involving a broad spectrum of endogenous (genetic and hormonal) and environmental factors. BMD is frequently used as a skeletal phenotype in studies evaluating the genetic background of osteoporosis (RIZZOLI et al. 1995). Although BMD is the most important predictor of fractures, results of recent studies (UITTERLINDEN et al. 1998, GRANT et al. 1996) have shown that the genes determining bone fragility and risk of fractures may be not identical with those associated with BMD. This fact shows the complexity of genetic determination of osteoporotic fractures (Fig.1)(RIZZOLI et al. 2001).

Genetic approaches

Numerous twin (POCOCK et al. 1987) and family studies (CUMMINGS et al. 1995) showed that genetic factors may account for up to 85 % of interindividual bone mass variance. BMD is a complex trait that does not exhibit classic Mendelian recessive or dominant inheritance attributable to a single gene locus (LANDER et al. 1994). There are few specific variants of low BMD representing a clinical expression of a mutation in a single gene – osteogenesis imperfecta (WILLING et al. 1990), osteoporotic syndromes due to mutations in the aromatase (MORISHIMA et al. 1995) or ESRα genes (SMITH et al. 1994) and osteoporosis-pseudoglioma syndrome (RALSTON et al. 2002). Besides these examples the heritability of BMD and osteoporosis in the population at large is certainly polygenic.

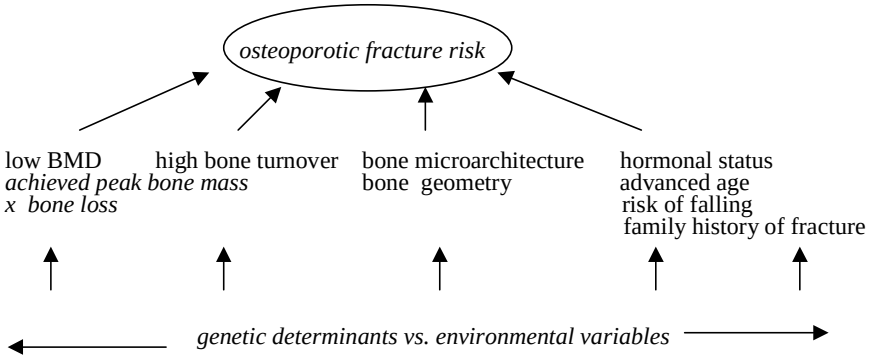


Figure 1. Interaction of genetic and non-genetic factors in a risk of osteoporotic fractures

Table 1.
Possible mechanisms how frequent polymorphisms may influence a phenotype

linkage disequilibrium with another trait-causing mutation in a nearby locus
effect on *mRNA stability*
effect on the *rate of gene transcription (amount of mRNA)*
modification of resulting *amino acids* sequence

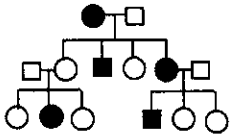
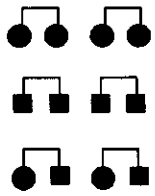
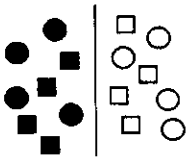
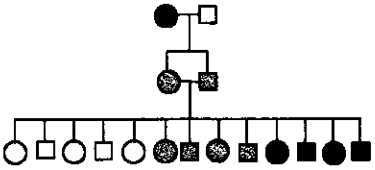
To look for the evidence of the genetic determination of osteoporosis, DNA markers – frequent sequence modifications, are examined for their potential association or linkage to a clearly defined skeletal phenotype. The DNA markers are represented by single nucleotide polymorphisms (SNP) or variable tandem repeats (microsatellites) spread through the genome or localized in the candidate loci or candidate genes. Regions of chromosome that contain alleles linked to a quantitative phenotypic trait such as BMD are called quantitative trait loci (QTL). DNA polymorphisms usually serve as DNA markers assuming that they lack functional significance, although in certain terms they might have some functional consequencies. Another possible explanation for positive associations found in candidate studies can be a linkage disequilibrium with other causal mutation exerting direct effect on phenotype (Gennari et al. 2001) (Table 1)**.** The main strategies employed in the studies of genetic basis of osteoporosis are discussed in more detail bellow and summarized in Table 2.

Linkage analysis dissects the inheritance of the disease or defined phenotype in relation to cosegregation of polymorphic genetic markers within a pedigree (Lander et al. 1994, Hobson et al. 2001). The linkage between the marker and inherited phenotype is evaluated by lodscore (logarithm of the odds that loci are linked rather than unlinked) (Stewart et al. 2000). It represents a model that is straightforward for simple Mendelian traits that can be complicated for complex traits. Furthermore, the use of the wrong model can lead to false linkage or more likely can miss it altogether (Lander et al. 1994).

Johnson et al. (1997) have linked a high bone mass phenotype to chromosomal locus 11q12-13 in a single extended pedigree suggesting the existance of a single major gene regulating BMD. Gong et al. (1996) have assigned osteoporosis-pseudoglioma syndrome to the same chromosomal region. However, a recent sibling pair study (Deng et al. 2001) has not confirmed the linkage between BMD and chromosomal region 11q12-13. Besides these results, the majority of reports have shown involvement of mul-

Table 2.
Different strategies to analyse the genetic contribution to complex disease such as osteoporosis

Genetic approaches in scheme and their strong and weak points

Linkage analysis		statistically robust model for simple Mendelian traits unclear mode of complex trait inheritance limited number of suitable families limited generalizability for population at large
Allele-sharing methods sib-pair analysis		nonparametric model-free method nonavaibility of affected sib pairs debatable in populational context
Association studies		easy to carry out evaluation in populational context risk of a false association- limitations difficult to control for confounding factors - - environmental, nutritional etc.
Experimental crosses in animals		large progeny genetic homogeneity elimination of envirnomental variables risk of discordant regulatory genes between different species

(based on Ralston et al. 2002, Lander et al. 1994)

tiple genes in BMD regulation (Devoto et al. 1998). Duncan et al. (1999) have carried out a candidate gene approach within the known candidate genes in families of probands with osteoporosis with a significant linkage documented between BMD and PTHreceptor marker (D3S1289). Seven other loci (e.g. IL-1, EGF, IL-4, ESR1, IL-6) have also shown some evidence for linkage.

Allele-sharing methods in sib pairs involve testing as to wheather affected relatives inherit a specific genetic region more often than expected under random Mendelain segregation (Lander et al.1994). As an example, allelic variations at the IL-6 locus showed evidence of linkage to decreased BMD in the sibling pair analysis of Japanese women (Ota et al. 1999). Similarly, a role of COLIA1 in determining BMD has been documented both by linkage and association analysis in dizygotic twin pairs and in a cohort of postmenopausal women (Brown et al. 2001). As proposed by Devoto et al. (1998) in a cohort of affected sib-pairs, several loci on chromosomes 1, 2 and 4 may harbor QTL related to low BMD.

In experimental animals interbreeding of mouse strains with high or low BMD allows the construction of a linkage model or allele-sharing study in a large genetically homogenous progeny with markedly varying bone mass. Complicating environmental factors are easily controlled. On the assumption that the main regulatory genes are shared between species, numerous genetic loci have been identified. Klein et al. (2001) carried out a genome wide link-

Table 3.
Limitations of association studies - a potential explanation for divergent results

population heterogeneity - admixture
small sample size
variability of environmental and nutritional factors
inappropriate case-control selection

age study in genetically distinct laboratory mouse strains derived from inbreeding of progenitors with high (C57BL/6) and low (DBA/2) BMD. QTL analysis has identified loci on chromosomes 1,2, 4 and 11 linked to peak bone mass.

Association studies represent the main model of human genetics of osteoporosis. Polymorphic markers are associated with a certain skeletal phenotype in the population assuming that the affected population may share markers more often than controls (Lander et al. 1994). Association studies have several limitations that can lead to false, artifactual relationships which cannot be replicated by others (Table 3) (Alonso et al. 1998). Very few polymorphisms have been also analysed in vitro to functionally explain the mechanism of how the polymorphisms alter bone metabolism at the molecular level.

Candidate gene studies

Association studies in osteoporosis focussed on genes encoding calciotropic hormones and their receptors, bone matrix proteins and local regulators of bone metabolism. The list of bone-related candidates that became a matter of association studies up to the present is indicated in Table 4 and some of them are reviewed below.

Vitamin D receptor gene. Polymorphisms of the vitamin D receptor (VDR) gene have become one of the most extensively investigated. Active vitamin D through VDR controls calcium homeostasis by regulating the target genes expressions. Morrison et al. (1994), reporting a significant association between BsmI polymorphism and BMD in a twin and population-based study, summarized that BsmI alleles may account for up to 75 % of genetic impact on bone thus rendering the VDR gene a major regulating gene in bone metabolism. Other polymorphims at the 3´end of the VDR gene – ApaI and TaqI- have also been described (Riggs et al. 1995). However, the following studies have brought conflicting results, either consistent (Fleet et al. 1995, Langdahl et al. 2000), inverse (Uitterlinden et al.1996) or negative (Kroger et al. 1995, Garnero et al. 1996, Aerssens et al. 2000) associations between VDR genotypes and bone mass to those originally reported by Morrison, indicating that the relationship between VDR polymorphisms and bone mass is rather weak. A meta-analysis of 16 separated studies have shown that BMD varied significantly in BsmI genotypes only at the hip and the difference seemed to decrease with age (Cooper et al. 1996) .

In exon 2, a coding VDR polymorphism (FokI) has been described that creates an alternative translation initiation start codon and thus in consequence changes the VDR protein structure by three aminoacids (Gross et al. 1996). A promising association between ff genotype and low BMD at the lumbar spine in a cohort of postmenopausal women has been published by Gross et al. (1996). Although the data provided by Harris et al. (1997) have documented a relation of FokI alleles to decreased BMD at the hip, subsequent studies have failed to demonstrate any asssociation (Eccleshall et al. 1998, Langdahl et al. 2000a, Ferrari et al. 1998). *In vitro* studies investigated the role of f-VDR and F-VDR variants in ligand or DNA binding affinity and in target gene transactivation (Arai et al. 1997, Gross et al. 1998), however, with inconsistent conclusions.

VDR polymorphisms have been found related to intestinal calcium absorption. The relationship is modified by a high or low calcium diet (Dawson-Hughes et al. 1995, Gennari et al. 1997). Association between the FokI genotypes and serum levels of

parathyroid hormone and dehydroepiandrosterone sulphate has been documented (Žofková et al. 2002a) allowing a tempting hypothesis that the genotype impact on bone regulation is exerted via hormonal pathways. Thus regulation of bone metabolism by VDR gene is, except for the direct skeletal action, mediated through extraskeletal pathways, such as calcium balance and hormonal status which might reflect functional consequences of VDR genotypes in different target tissues.

The VDR alleles have been analyzed in relation to osteoporotic fractures with mixed results (Berg et al. 1996, Feskanich et al. 1998), although in a large cohort of postmenopausal women VDR and COLIA1 interlocus interaction has showen a strong relation to osteoporotic fractures independently of BMD (Uitterlinden et al. 2001). VDR genotypes have been involved as well in individual response of BMD to etindronate (Marc et al. 1999) and vitamin D therapy (Graafmans et al. 1997).

Furthermore, the VDR gene, similarly to other bone-related candidates, appears to be associated with a risk of a number of apparently distant diseases including inflamatory and degenerative disorders or tumours (Table 5), which is hypothesized on the basis of the linkage disequilibrium with a nearby causal gene.

Calcium-sensing receptor gene. By coupling the extracellular calcium levels to intracellular signalling pathways that modify parathyroid hormone or calcitonin secretions and calcium reabsorption in the kidney, calcium-sensing receptor (CASR) contributes to mineral ion homeostasis (Hendy et al. 2000). As known mutations of the CASR genes alter the extracellular ionised calcium set-point and are responsible for familiar hypercalcaemia or hypocalcaemia (Cole et al. 1999), genetic polymorphisms of CASR have been examined for their potential involvement in a predisposition to various bone metabolic disorders such as osteoporosis (Pearce et al. 1997). The cytosine-adenine (CA) repeat polymorphisms at the human CASR gene has been associated with BMD in Japanesee postmenopausal women (Tsukamoto et al. 2000a).

The carriers of 18 CA repeats had significantly lower BMD of radial bone than others. Recently, another CASR gene polymorphism A986S has been analysed in relation to low postmenopausal (Takacs et al. 2002) and premenopausal BMD (Eckstein et al. 2002). Whereas the first study has not found a significant relationship, in the second study heterozygous genotypes prevailed in women with low BMD than in healthy controls. Although this kind of association is not a unique finding, a clear allele-dosage effect on BMD in heterozygotes is missing which makes the pathogenetic understanding difficult.

Calcitonin and calcitonin receptor genes. Another calcium-regulating hormone, calcitonin, inhibits osteoclastic bone resorption and stimulates urinary calcium excretion through calcitonin receptor (CALCR) that belongs to a family of G protein coupled membrane receptors. Genetic polymorphisms of calcitonin and CALCR have been investigated in relation to BMD, as they may play a role in the pathogenesis of osteoporosis. In the CT gene a dinucleotide polymorphism has been investigated in a cohort of postmenopausal women (Miyao et al. 2000a). Probands who had 10 cytosine-adenine repeats had significantly lower total BMD than women who carried CA repeats of different length. In the CALCR a cytosine/thymine polymorphism at position 1377 that changes 463th aminoacid in the fourth intracellular domaine of the receptor has been identified in the Japanese population (Nakamura et al. 1997). In comparison with Asian population the prevalent allele in Caucasian women was T allele and the TT genotype has been associated with significantly lower BMD at the lumbar spine in Italian postmenopausal women (Masi et al. 1997). On the contrary, in a large cohort of postmenopausal women from north-eastern Italy lower BMD at the lumbar spine has been associated with CC genotype (Braga et al. 2000). In a subset of younger postmenopausal women CC genotype was also related to decreased BMD at the hip, thus suggesting that the CALCR genotypes might influence the process of acquiring peak bone mass rather than the process of bone loss. To point out the contraversy, in the study of Taboulet et al. (1998) the presence of both alleles of the receptor has been revealed as the more advantageous because higher BMD at the femoral neck has been observed in heterozygous subjects. This issue needs further investigation in varied populations to definitely conclude which allele (T/C) in CALCR is favourable and vice versa in postmenopausal bone mass regulation.

Table 4.
The overview of candidate gene polymorphisms related to bone metabolism in association studies

Candidate Genes	Polymorphism Characteristics	References
A. Calciotropic hormones and receptors		
Vitamin D receptor (VDR)	5′ FokI	Gross et al. 1996
	3′BsmI, ApaI, TaqI	Morrison *et al*.1994, Riggs *et al*.1995
Estrogen receptor (ESR)-alpha	5′ PvuII, XbaI	Kobayashi *et al*. 1996
	BstUI (G/C)	Han et al., 1997
	codon 325 (C/G)	Jurada *et al*. 2001
	5′ TA repeat	Sano et al., 1995
Estrogen receptor (ESR)-beta	CA repeat (18-32)	Ogawa et al., 2000
Calcitonin receptor (CALCR)	AluI (C/T - Pro/Leu)	Taboulet et al.,1998
	TaqI	Masi et al., 1998
Calcitonin	CA repeat (10-20)	Miyao *et al*. 2000
Parathyroid hormone	BstB1	Hosoi *et al*. 1999
Glucocorticoid receptor	A/G - Asp/Ser (exon2)	Huizenga et al., 1998
Androgen receptor	(AGC)n repeat (exon1)	Sowers *et al*. 1999
Calcium sensing R	CA repeat (12-20)	Cole et al.,1997
	A986S	Cole et al. 1999
B. Bone matrix components		
Collagen typeI alpha1 (COLIA1)	Sp1 (intron1)	Grant e*t al*. 1996
	MnII	Willing et al., 1998
	RsaI	Baker et al., 1991
Collagen typeI alpha2 (COLIA2)	PvuII	Willing et al., 1998
	RsaI	Willing et al., 1998
	(ACT)n VNTR (intron12)	Pepe et al., 1993
Osteocalcin	HindIII, C/T (promotor)	Dohi et al., 1998
Osteonectin	CA repeat	Willing et al., 1998
Osteopontin	CA repeat	Willing et al., 1998
alpha2-HS-glycoprotein	AHSG1/AHSG2 (ISE)	Zmuda et al., 1998
matrixGlaprotein	CA repeat (13-18)	Tsukamoto et al. 2000b
C. Local regulators		
TGF-beta1	713-delC (intron 4), C/T(Thr/Ile, exon 5)	Langdahl et al., 1997
	T/C - Pro/Leu (exon1)	Yamada et al. 1998
IGF-1	CA repeat (promotor)	Miyao *et al*. 1998
IL-1beta	C/T (exon 4)	Langdahl et al. 2000b
IL-1-receptor antagonist	86 VNTR (intron2)	Keen et al. 1998, Langdahl et al. 2000
IL-6	3′AT repeat	Murray *et al*. 1997
	G/C (promotor)	Ferrari *et al*. 2001
	CA repeat (13-18)	Tsukamoto *et al.*1998
TNF-receptor R2	repeat-intron 4, VNTR 3′	Spotila *et al*. 2000
Bone morphogenetic protein (BMP-4)		Semprini et al., 2000
D. Miscellaneous		
Methylentetrahydrofolate reductase (MTHFR)	MTHFR A/V	Miyao et al. 2000b
Apolipoprotein E	E2, E3,E4	Shiraki *et al*. 1997
Collagenase	BanI (A/T)	Thiry-Blaise *et al*. 1995
CYP 19 aromatase	(TTTA)n repeat intron 4, C/T exon 10	Gennari et al., 2000
Peroxisome proliferator-activated receptor gamma gene (PPARgama)	silent C/T (exon6)	Ogawaet al., 1999
VitD - binding protein	(TAAA)n intron 8	Papiha *et al*.1999
beta2 adrenergic R	(Trp/Arg)	Matkovic et al., 1997
Werner helicase gene	T/C (Cys/Arg)	Ogata *et al*. 2001

Bone-related candidate gene polymorphisms predict a risk for other diseases than osteoporosis

Candidate gene	Disease	References
Apolipoprotein E	Alzheimer dementia, cardiovascular disease	Corder et al. 1993, Davignon et al. 1988
VDR (BsmI, ApaI, TaqI)	sporadic primary hyperparathyroidism	Carling et al.1995
VDR (FokI, BsmI, ApaI)	Grave´s disease	Ban et al. 2000
VDR (BsmI)	osteitis fibrosa in uremic patients	Nagaba et al. 1996
	osteoarthritis	Uitterlinden et al. 1997
	breast cancer	Ingles et al. 2000
	benign prostate hypertrophy	Habuchi et al. 2000
	prostate cancer	Habuchi et al. 2000
	IDDM	McDermott et al. 1997
	NIDDM	Hitman et al. 1998
	multiple sclerosis	Fukazawa et al. 1999
	HIV-1 disease progression	Velasco et al. 2001
	mortality risk in hemodialysis patients	Fibla et al, 2001
VDR (polyA, BsmI, TaqI)	colon cancer	Slatter et al. 2001
ESR-alpha (TA repeat)	autoimune thyroid disease	Ban et al. 2000
	rheumatoid arthritis	Takagi et al. 2000
	hypertension	Lehrer et al. 1993
ESR - alpha (PvuII, XbaI)	breast cancer	Hill et al. 1989
TGFbeta1	spinal osteophytosis	Yamada et al. 2000

Estrogen alpha receptor gene. C/T transition in the coding region of the estrogen alpha receptor (ESRα) led to a partial estrogen resistance with low BMD as a phenotypic expression in a young man (Smith et al. 1994), which has drawn two important conclusions – mutations in ESR may not be lethal and estrogens are important for bone mass regulation in both genders. Two single nucleotide polymorphisms in the first intron recognized by restriction enzymes PvuII and XbaI have been associated with low BMD in Japanese postmenopausal women (Kobayashi et al. 1996). Following studies in different populations have pointed out the impact of ESR1 genotypes on the acquisition of peak bone mass, while others have demonstrated the relationship to postmenopausal bone loss (Rizzoli et al. 2001). A dinucleotide repeat has been described in the regulating region of the ESR gene with a positive association to BMD both in the Japanese and American population (Stewart et el. 2000). However, as in other polymorphisms, negative results have been also published (Vandenvyer et al. 1999).

Insulin-like growth factor I gene. Not only for neonatal development of the skeleton and growth in childhood insulin-like growth factor I (IGF-1) is essential, but it plays an important role also during adulthood in regulation of cortical and trabecular bone formation (Rosen et al. 1995). This factor exerts a pleiotropic anabolic effect; it stimulates both proliferation and differentation of osteoblasts with osteocalcin production, and increases type I collagen synthesis (Schmidt et al. 1992). Low serum IGF-1 levels are strongly associated with an increased risk of osteoprotic fractures (Garnero et al. 2000). While no association has been found between cytosine-adenine repeat polymorphism in the IGF-1 gene and BMD in Japanese postmenopausal women (Miyao et al. 1998), it has been documented in a cohort of postmenopausal Korean women. The presence of a 194-bp allele has been related to either higher BMD or to increased levels of circulating IGF-1 and was less frequent in the group of osteoporotic women in comparison with normal controls (Kim et al. 2002). The same microsatellite polymorphism has been found prevalent in a group of male patients with idiopatic osteoporosis more than in normal controls suggesting that IGF-1 gene may be a strong candidate also for male osteoporosis (Rosen et al.1998).

Collagen type I alpha1 gene. A relation of Sp1 polymorphism in collagen type I alpha1 gene (COLIA1) to osteoporosis seemed to provide a parallele with coding mutations of COLIA1 and COLIA2 in osteogenesis imperfecta (Willing et al. 1990, Spotila et al. 1991). Grant et al. (1996) have document-

ed that unfavourable Ss and ss genotypes are associated with low BMD and prevail in an osteoporotic fracture group compared to normal controls. Recent studies (Uitterlinden et al. 1998, Weichetová et al. 2000) have documented that Sp1 genotypes predict the risk of osteporotic fractures regardless of BMD, suggesting that polymorphic changes in a COLIA1 gene may reflect changed bone quality more than bone mineral content. However, as with other polymorphisms, these findings have not been confirmed by others (Liden et al. 1998). The molecular mechanism might be mediated through increased binding activity to the Sp1 factor leading to overproduction of alpha 1 chain with a relative lack of alpha 2 chain resulting in alpha1 homotrimer with impaired structural quality (Grant et al. 1996, Mann et al. 2001).

Recent studies have emphasized the need to look out for genes directly linked to fractures as some reports have shown that the risk of fractures persists after adjustment for BMD (Uitterlinden et al. 1998, Weichetová et al. 2000). However, the phenotype requires a strict definition considering the fact of phenocopy – admixture of traumatic fractures caused by environmental factors with fractures related to genetically disrupted bone quality (Nguyen et al. 2000). Thus BMD, as a typical quantitative trait, with parameters of bone quality and turnover, remains an important surrogate phenotype for genetic studies of osteoporosis.

Werner helicase gene. Low BMD, a common age-related disorder in normal postmenopausal women, is a part of phenotype in patients with Werner syndrome (WS) – a rare autosomal recessive progeroid syndrome characterised by the premature onset of multiple disorders (Salk et al. 1982). A thymine/cytosine polymorphism in a Werner helicase gene (WRN) responsible for WS has been examined in relation to BMD in Japanesse postmenopausal women (Ogata et al. 2001). Carriers of C allele exhibited lower BMD at the lumbar spine, thus indicating that the WRN gene may be another candidate for genetic regulation of osteoporosis. It would be of value to replicate the original observations in studies of other populations.

Apolipoprotein E gene. Variations in apolipoprotein E (ApoE) locus have been associated with a number of involutional disorders such as cardiovascular disease (Davignon et al. 1988) and Alzheimer dementia (Corder et al. 1993). Recently some studies have examined whether ApoE 4 allele might play a role in the pathophysiology of osteoporosis (Shiraki et al. 1997; Cauley et al. 1999; Muhlen et al. 2001). The underlying mechanism might be mediated through vitamin K homeostasis that contributes to carboxylation of osteocalcin (Saupe et al. 1993) or through bone tisssue ischemia in atherosclerotic vessels (Vogt et al. 1997). A recent report has indicated a possible involvement of circulating sex steroids in ApoE and bone mass relationship (Žofková et al. 2002b).

The contraversy regarding associations between genotypes and BMD may be explained in part by gene-gene and gene-environment interactions. Willing et al. (1998) have verified that BsmI polymorphism is related to BMD only in combination with allelic variations in ESR locus. Moreover, Deng et al. (1998) have found positive correlations between VDR and ESR genotypes, bone loss and bone response to hormonal therapy in postmenopausal women.

DNA chips and genetics of complex diseases. Microarray(biochip)-based technologies promise to provide an extensive view of biological information on the interplay of genes in the entire genome (Ott et al. 2000). Hybridization of RNA or DNA-derived samples to DNA chips allows to analyse either gene expression or the occurence of polymorphisms (Gerhold et al. 1999). DNA microarray technology holds significant promise to identify further genes predisposing to complex disease.

In conclusion, osteoporosis has a strong genetic component. Linkage studies in man and mice and association studies in different populations have analysed a number of candidate gene polymorphisms in relation to BMD, biochemical markers of bone turnover, ultrasound properties of bone, osteoporotic fractures and response to anti-osteoporotic therapy. However, the relevant and functional polymorphic marker has not been found yet that might reflect the complex and polygenic nature of BMD where the overall effect of an individually analysed polymorphism is low. The disparity of the observations also outlines the limitations of association studies leading to inconsistent and incomparable data. Only extensive studies in well-defined and homogenous populations that analyse the genetic markers in determination of either the peak bone mass or the rate of bone loss

and restricted to certain skeletal sites with elimination of confounding factors might bring insight to heritability of osteoporosis. In vitro experimental investigations are warranted to functionally analyse the underlying molecular mechanism implicated in the interaction of genotype and phenotype. Altogether, searching for the genes involved in bone metabolism remains a challenging task: to better understand the pathophysiology of the disorder, to identify individuals at risk and to target the particular treatment.

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