

Review Article

Pharmacogenomics of osteoporosis: Opportunities and challenges

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Abstract

The genetics of osteoporosis can be considered in two broad areas: disease susceptibility and drug activity. While the former has been studied, the latter is still largely untouched. Pharmacogenomics is the utilization of genetic information to predict outcome of drug treatment, with respect to both beneficial and adverse effects. The pharmacotherapy of osteoporosis is characterized by variability in therapeutic response with limited prediction of response on a patient-by-patient basis. This is particularly problematic in a clinical situation where therapy is typically required for several years before outcomes can be evaluated for an individual. Thus, the emerging field of pharmacogenomics holds great potential for refining and optimising pharmacological treatment of osteoporosis. Key components for future development of the pharmacogenomics of osteoporosis should include improved understanding of mechanisms of drug action, identification of candidate genes and their variants and expansion of clinical trials to include genetic profiling. This approach could provide clinicians and scientists with powerful tools to dissect novel molecular pathways involved in osteoporosis and to identify new drug targets. The iterative combination of innovative genomics with classical endocrinological approaches in osteoporosis research can be examined as a model of biological research and innovate therapeutical approaches in a continuing interaction between clinical science and basic research.

Keywords: Osteoporosis, Fracture, Bone Mineral Density, Vitamin D Receptor Gene, Collagen I alpha-1 gene, Pharmacogenetics

Background

Osteoporosis, the most common and serious skeletal "disease", is highly prevalent among the elderly of both sexes, with a combined lifetime risk for hip fracture, forearm fracture and vertebral fractures coming to clinical attention being around 40%, equivalent to the risk for cardiovascular disease¹. In the United States alone, osteoporosis affects 25 million people, and incurs estimated costs of \$60 billion. The lifetime risk of hip fracture, the most serious fracture, in Caucasian women is one in six; somewhat higher than the risk of diagnosis of breast cancer (one in nine)². Mortality is a frequent occurrence in the

months immediately following a hip fracture³. Fifty percent of surviving individuals will need help with daily living activities, and 15 to 25 percent will need to enter a long-term care institution shortly after the fracture³. Moreover, all major osteoporotic fractures are associated with a two- to three-fold increased mortality in both men and women³.

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue resulting in increased bone fragility and susceptibility to fracture. Bone mass is traditionally quantified by bone mineral density (BMD). At any specific skeletal region, BMD can be accurately and reliably measured by dual X-ray absorptiometry (DXA); as the amount of mineral per area of bone imaged.

Although fracture is the clinically relevant endpoint of osteoporosis, BMD is a primary predictor of fracture risk^{4,6}. Each standard deviation decrease in BMD is associated with a two-fold increase in the fracture risk⁷. The BMD – fracture relationship generally applies across the skeleton, with some site-specificity; i.e., hip fracture risk is more related to BMD measurements at the hip than lumbar spine or forearm. The relationship between fracture risk and BMD measurements

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is comparable to the relationship between stroke and blood pressure readings. In the same way that "hypertension" relates to cut-off value for blood pressure measurements, osteoporosis is based on a value for BMD below a cut-off threshold. Moreover, as for blood pressure, there is no threshold of BMD that discriminates absolutely between those who will or will not have a clinical event. Hence, a "normal" BMD measurement is no guarantee that fracture will not occur; only the risk is relatively low. Conversely, if BMD is in the osteoporotic range, then fractures are more likely, but still may not occur. At age 50 years, the proportion of women with osteoporosis who will fracture their hip, spine, forearm or proximal humerus in the next 10 years – i.e., positive predictive value – is about 45%. The detection rate for these fractures (sensitivity) is lower, i.e., a significant proportion of fractures occur in women above this BMD threshold.

BMD is not the only predictor of fracture; rather, it is the interaction of increased force, i.e., in falls, and decreased bone "strength". Although falls prevention may influence osteoporotic fracture prevention, determinants of bone "strength" have been the major focus to date with many treatments being developed and applied effectively clinically. BMD is a valuable predictor of future fracture risk, and partly accounts for bone size, but not bone structure, including mass distribution and quality, which are considered to contribute to bone strength. Quantitative ultrasound measurements, broadband ultrasound attenuation (BUA) and speed of sound (SOS), have been proposed as additional measures of bone quality, related to trabecular connectivity and bone matrix. Moreover, many clinical studies have supported a role for normalization of elevated bone turnover as part of the anti-fracture efficacy of the widely used anti-resorptive therapies.

Osteoporosis is, therefore, a complex disease. Its complexity is not just characterized by the multiplicity of clinical aspects, but also multiple determinants. Like many other multifactorial diseases, osteoporosis is determined by environmental factors, by genetic susceptibility and likely by the interaction between these factors. Genetic variations do not necessarily cause osteoporosis or fracture, but they can influence a subject's susceptibility to specific environmental factors and so modify the disease risk. This implies that each subject in the population has a unique risk profile that can change with time. Hence, population data can be only cautiously extrapolated to the individual subject. Yet, at present, decisions about diagnosis and treatment of osteoporosis are still based on statistical data of the subjects' general population. Clearly, this generalized average approach is suboptimal compared with an individualized approach, according to individual genetic and environmental risk profile. Osteoporosis presents an ideal case for such an approach, because of its strong genetic precipitation and high variability in the susceptibility of fracture risk among individuals. In this framework, the principles of pharmacogenomics, which seek to correlate phenotypes and biomarkers by taking advantage of genomic technology, could be applied to identify the actual genetic basis of inter-individual variation in drug efficacy.

Genetics of osteoporosis

Heritability of fracture. Data on heritability of fracture *per se* are scarce. In a Finnish twin study, approximately 35% of the variance in the liability to fracture (in both males and females) was attributable to genetic factors⁸. In a recent family study, approximately 25% of the liability to one fracture type, i.e., Colles' fracture of the wrist, was attributable to genetic factors⁹. Familial analysis within the Study of Osteoporotic Fracture¹⁰ suggests that women, whose mother had had a hip fracture, had a two-fold increase in risk of hip fracture compared with controls. The risk of hip or other fractures was three-fold higher with a paternal history of wrist fracture. In two small studies of osteoporotic women with vertebral or hip fractures, their daughters had bone density deficits intermediate between their mothers and "expected" at the site of their mothers' fracture i.e., lumbar spine or proximal femur^{11,12}. Similar observations have been made in both elderly men and women¹³.

Heritability of bone mineral density. Most genetic studies on osteoporosis have focused on the predictive bone phenotypes, such as BMD. Although BMD is determined by both environmental and genetic factors, it has been estimated from twin studies that 70% to 80% of variance of BMD measured at the lumbar spine and femoral neck is attributable to genetic factors in twin samples¹⁴⁻¹⁸. Heritability of forearm BMD appears to be lower than that in either the femoral neck or lumbar spine^{17,18}. In these studies, there is evidence for pleiotropic effects, i.e., BMD in various skeletal sites being determined by both common and site-specific sets of genes^{16,21}.

Genetic influence on bone turnover. Change in BMD during adult life is the result of the net imbalance between bone formation and bone resorption. These are typically assessed by measurements (in blood or urinary excretion) of various products of osteoblast (bone formation) and osteoclasts (bone resorption) cell activity. Indices of bone formation include osteocalcin, bone-specific alkaline phosphates, procollagen I carboxy-terminal and amino-terminal propeptides. Indices of bone resorption include urinary excretion of hydroxyproline or more specifically pyridinoline cross-links, and more recently, urinary type I collagen cross-linked N-telopeptides and urinary or serum type I collagen C-telopeptide breakdown products. Genetic factors have been shown to contribute significantly to the inter-individual variance of bone formation markers (both osteocalcin and collagen C-terminal propeptide of type 1 collagen) in premenopausal twins¹⁹⁻²¹.

Heritability of quantitative ultrasound (QUS). The genetic influence on different types of QUS measurements, namely BUA and SOS, has been shown to be 0.53 to 0.82²². BUA measurements have been reported to be more strongly correlated between mothers and their postmenopausal rather than their premenopausal daughters; i.e., the reverse of what has been reported for DXA measurements^{23,24}. These observations suggest that different genetic influences

act on components of the bone phenotype as measured using QUS and DXA. There are no data on heritability of speed-of-sound measures along cortical bone. Genetic correlations observed between transmission QUS and BMD measurements have been moderate, 0.32 to 0.59²². Thus, genes that influence variation in BMD might, but not necessarily, influence variation in QUS, and vice versa. This is consistent with QUS measuring additional non-density characteristics of bone. In any case, a significant part of the variability of QUS and DXA BMD measurements appears unrelated, consistent with their assessment of some distinct bone phenotypic characteristics.

The recognition that various bone-related traits are largely determined by genetic factors has led to an intensive search for specific genes either linked or associated with these traits. Gene-search studies have focused on bone mineral density using the two major approaches of genome-wide screening and candidate genes. The candidate gene approach is based on *a priori* knowledge of the potential function of the gene involved, and takes advantage of the relevant and known biochemical pathway of bone physiology. Based on this commonly used approach, currently 16 genes have been proposed as potential candidates for bone mineral density, including vitamin D receptor, collagen type I α 1, osteocalcin, IL-1 receptor antagonist, calcium sensing receptor, α 2HS glycoprotein, vitamin D binding protein, osteopontin, osteonectin, estrogen receptor α , interleukin-6, calcitonin receptor, collagen type I α 2, parathyroid hormone, and transforming growth factor α 1. As well as the above gene polymorphisms, other polymorphisms in genes including other steroid receptor, cytokine, and bone matrix proteins genes, and more "distant" osteoporosis candidate genes such as apolipoprotein E have also been suggested^{25,44-50}. A feature of these candidate gene studies has been the wide range in positive and negative outcomes and, even in consistent studies, the wide range of effect sizes.

The candidate genes identified so far have been neither strong nor consistent enough to have major clinical predictive value. More importantly, since their relationship with bone biology was the basis of their initial study as "candidates", none could provide novel targets for development of new therapies. It seems likely that minor variations in the regulation or function in several genes, each making relatively small contributions, interact to make up the genetic component of osteoporosis. Under this scenario, individual studies seeking to establish an association between a candidate gene and markers of the disease may yield spurious results. The best strategies for resolving the genetic and environmental contributions to such a polygenic disease such as osteoporosis are not clear.

The less common alternative approach of genome-wide scan has yielded interesting findings. By using linkage analysis of data from a family with osteoporosis-pseudoglioma syndrome (OPS), a disorder characterised by severely low bone mass and eye abnormality, investigators were able to localise the OPS locus to chromosomal region 11q12-13⁵¹. At

the same time, a genome-wide linkage analysis of an extended family with 22 members among whom 12 had very high bone mass (HBM) suggested that the HBM locus also located within 30cM region of the same locus⁵². In follow-up studies using the positional candidate approach both research groups found that a gene encoding the low-density lipoprotein receptor-related protein 5 (LRP5) was linked to both OPS and high bone mass^{53,54}. The finding that LRP5 gene is linked to high bone mass was subsequently confirmed in a family study which included individuals with exceptionally high BMD but were otherwise phenotypically normal⁵⁵. This study showed that a missense mutation (G171V) was found in high-BMD individuals⁵⁵. A recent family study further identified six novel mutations in LRP5 among 13 confirmed polymorphisms that were associated with different conditions with increased BMD⁵⁶. The conditions included endosteal hyperostosis, Van Buchem disease, autosomal dominant osteosclerosis, and osteopetrosis type I. The association between LRP5 and BMD has also recently been shown in a general unselected population⁵⁷.

An example of the success of the combined linkage and association strategy was demonstrated in a recent study in 207 nuclear families with 1323 individuals of Icelandic ancestry. In this study, by first performing a genome-wide scan in the families, the BMP2 gene was found to be linked to the variation in BMD; and by association analysis, with increased fracture risk and BMD⁵⁸.

Pharmacology of osteoporosis

In the past decade there have been remarkable advances in the understanding of basic bone biology leading to targeted approaches both in the prevention and effective treatment of osteoporosis. Among the approved pharmacological therapies for osteoporosis, estrogen replacement therapy, selective estrogen receptor modulators (SERMs), calcitonin, vitamin D derivatives, potent bisphosphonates, and parathyroid hormone have been introduced to clinical use⁵⁹. Most of these drugs, with the exception of parathyroid hormone, act as anti-resorptive agents, and thus decrease bone loss.

Paradoxically, although these agents have modest effects of increasing bone density, they exert a much greater effect on fracture risk reduction. For example, alendronate (a potent bisphosphonate) can improve BMD by 4 to 8%, which based on the BMD-fracture relationship, could be expected to reduce fracture risk by 12 to 28%; but in reality, the fracture risk among alendronate-treated patients was reduced by about 50%. Potential mechanisms for this higher-than-BMD-expected reduction are still being evaluated.

A common feature of all clinical trials involved pharmacological intervention in osteoporosis is that the efficacy and safety are highly variable among patients, such that the range of response is considerable, ranging from "success" to little or no response. For example, the standard deviation of change in BMD induced by potent bisphosphonates is more than twice the rate of change⁵⁹. As a result, while the major-

ity of patients experience an increase in BMD, a small proportion (perhaps 5 to 10%) of patients apparently still lose bone. Thus, although very few patients experience absolutely no therapeutic effects following typical anti-resorptive treatment, no treatment currently prevents all fractures. Moreover, some subjects experience significant adverse effects. These effects, both positive and negative, are experienced over years. Thus, while these treatments are overall beneficial, no reliable means exist to predict who will experience unfavourable or an adverse effect of some type.

Pharmacogenomics

On the clinical level, drug response is affected by many factors, including age, sex, ethnicity, and concomitant disease or drug therapy. However, it is also possible that genetic factors affect the variability in drug response⁶⁰. Indeed, evidence of genetic influence on inter-subject drug response had been reported as early as the 1940s, in the case of peripheral neuropathy in a substantial number of patients treated with the anti-tuberculosis drug, isoniazid⁶¹. It was also observed that African-American soldiers given antimalarial drugs were more likely than their Caucasian colleagues to develop haemolytic anaemia, due to inherited metabolic enzyme differences⁶². Further evidence of genetics of drug response was found in twin studies, in which identical twins were more similar than non-identical twins in regard to the plasma half-life of numerous drugs, providing the best experimental indication of strong genetic components in drug elimination⁶³. Thus, genetic factors may determine an individual's response to pharmacological therapy of osteoporosis and their susceptibility to adverse drug reactions, for each specific drug. Although drug elimination and metabolism can be relatively easily studied, other components that influence drug effects are either unknown or are more difficult to study.

On the molecular level, pharmacological agents act by interacting with proteins such as receptors, enzymes and intracellular signalling proteins. Therefore, when a drug is taken, its absorption, distribution, excretion and pharmacological responses are likely determined by the interactions among those factors, including carrier proteins, transporters, metabolising enzymes, receptors, and co-factors. Members of the cytochrome P450 (CYP), including CYP2D6, 3A4/3A5, 1A2, 2E1, 2C9, and 2C19 are known to influence drug efficacy and toxicity^{64,65}. For example, patients who are homozygous for the CYP2D6 null alleles exhibit a poor metabolizer phenotype, with impaired degradation and excretion of many drugs, including debrisoquine, metoprolol, nortriptyline, and propafone⁶⁴. These poor metabolizers are more likely to exhibit adverse drug reactions. The frequency of this recessive trait ranges from 1% to 2% in Asians, 5% in African Americans and up to 6% to 10% in Caucasian populations⁶⁶⁻⁶⁹. Similarly, patients who are homozygous for the "null" allele of the P450 isoform CYP2C19 are highly sensitive to omeprazole, diazepam, propranolol, mephentoin, amitriptyline, hexobarbital and

other drugs⁶⁴. The CYP2C19 poor metabolizer phenotype comprises 2% to 5% of Caucasians and 3% to 23% of Asians, resulting largely from a single base pair mutation (A→G) in exon 5 of the coding region⁷⁰. Another polymorphically expressed member of the cytochrome P450 family, CYP2C9, metabolizes ibuprofen, naproxen, piroxicam, tetrahydrocannabinol, phenytoin, tolbutamide, and S-warfarin⁷¹; some of which have narrow therapeutic indices. Amino acid substitutions at codons 144 and 359 in the coding region of CYP2C9 result in a 5-fold decline in metabolic activity. Although the frequency of these 2 allelic variants is uncertain, approximately 25% of Caucasians appear to be heterozygous for one or the other variant, leading to a predicted frequency of 5% for the compound homozygous genotype⁷².

Pharmacogenomics of osteoporosis

Some drugs used in osteoporosis therapy, bisphosphonates for example, are not subject to metabolism, but many others are metabolized to active components or as part of their elimination pathway. Despite the evidence of genetic effects on the variation in efficacy and safety of pharmacological agents in other diseases, these are still largely untested in the treatment of osteoporosis, but their potential is underlined by their rapid adoption in disciplines such as obesity and hypertension.

Nevertheless, recent evidence suggests that genetic factors may mediate the response to drug treatment⁷³, and modify the dynamic association between bone turnover markers and bone density. A recent series of studies by Palomba and colleagues⁷⁴⁻⁷⁶ suggested that among postmenopausal women who were on alendronate and hormone replacement therapy (HRT) treatments, the *b* allele of the VDR's Bsm-I polymorphisms was associated with a greater increase in BMD than those carriers of the *B* allele. However, interestingly, among patients on RLX the *B* allele carriers were associated with a greater increase in BMD than the *b* allele carriers. As a result of the opposite effects, among those on combined ALN and RLX there was no significant association between VDR polymorphisms and BMD change. These results clearly illustrate the interaction between VDR polymorphisms and various anti-resorptive drug therapies in BMD change.

In a study of 21 premenopausal Caucasian women who were homozygous for the VDR genotypes (*BB* or *bb*), it was found that baseline osteocalcin, 1,25-(OH)₂D, type I collagen carboxyterminal telopeptide, and inorganic phosphate levels were significantly higher and spinal bone mineral density was significantly lower in the *BB* allelic group. However, after calcitriol administration, similar serum levels of 1,25-(OH)₂D were attained in both genotypic groups. The increase in serum osteocalcin levels in the *BB* group was significantly less than that in the *bb* group. The genotype-related baseline difference in osteocalcin levels was not apparent at similar serum 1,25-(OH)₂D levels. By contrast, baseline differences in phosphate and type I collagen carboxytermi-

nal telopeptide persisted throughout the study. Moreover, parathyroid hormone was less suppressed in the low bone density group despite similar ionized calcium levels⁷⁷.

The VDR gene polymorphisms may also affect the dynamic association between dietary calcium intake and bone density. For example, on lower dietary calcium intakes, gut calcium absorption in women with VDR's *BB* genotype did not increase, but those with *bb* genotype did^{78,79}. The difference in gut calcium absorption between the two alternate homozygotes for the vitamin D receptor start codon polymorphism was 42%⁸⁰. As with other studies of bone and genetics, a number of studies have found positive relationships between the vitamin D receptor gene alleles and calcium homeostasis⁸⁰⁻⁸², while other studies have been negative⁸³⁻⁸⁶.

Similarly, longitudinal studies have also shown differences of the bone density response to calcium intake according to vitamin D receptor genotype. In one study, the vitamin D receptor heterozygotes responded to calcium intake while the alternate homozygotes either gained or lost bone irrespective of calcium intake⁸⁷. By contrast, in a second study, the *BB* homozygotes gained some bone when supplemented from a very low basic calcium intake⁸⁸.

However, despite apparent differences in gut calcium absorption, a number of studies have not found any difference in intestinal vitamin D receptor level^{86,89,90}. By contrast, differences in parathyroid gland regulation have been related to vitamin D receptor polymorphisms^{91,92}.

Another potential gene-environment interaction for the vitamin D receptor gene would be in relation to simple vitamin D itself or the active hormonal forms of vitamin D. Differences in response of bone density to the vitamin D metabolites and analogs have been reported according to the vitamin D receptor genotypes, particularly in Japanese studies^{93,95}. The more common *bb* genotype in Japanese cohorts (about 75% of the subjects) was more responsive compared with the heterozygotes, who did not respond well or actually worsened. Given that the heterozygote is the most common genotype in most Caucasian groups, these differences have an intriguing parallel to the differences that have been observed in response to the active vitamin D compounds in clinical studies of osteoporosis between Japanese and Caucasian groups. In another study, the response to simple vitamin D varied according to vitamin D receptor genotype⁹⁶.

The mechanism by which any changes in the vitamin D receptor alleles may account for changes in calcium and bone homeostasis is not clear. At a simple level it is possible that there may be subtle differences in the regulation of the gene or in stability of the mRNA product. Some initial *in vitro* studies suggested that change in stability of mRNA product^{37,97}; however other studies do not confirm this effect⁹⁸⁻¹⁰⁰. Another mechanism may relate to changes in alternative transcripts from the recently reported multiple promoters of single human vitamin D receptor gene¹⁰¹.

The differences in the vitamin D allelic effects may relate to genetic backgrounds and/or environmental factors such as calcium and vitamin D intakes. Genetic backgrounds may

relate to other allelic gene effects, e.g., the estrogen receptor genotype^{102,103}. By this mechanism, allelic effects could differ between environments. Some effects could be quite unexpected as, for example, the apparent protection against some chronic infections reported in a recent African study¹⁰⁴ and relationship to risk of osteoarthritis of spine and hip¹⁰⁵⁻¹⁰⁷.

Elucidating pharmacogenomic mechanisms

Currently, most pharmacogenomic studies depend on comparing expression profiles at the mRNA (genomics) or protein (proteomics) level for a given tissue or cell type after a relevant stimulus. Comparison of expression profiles at the mRNA level is attractive, particularly with the advent of recent availability of microarrays allowing concurrent analyses of tens of thousands of genes. This new technology can rapidly genotype individuals to provide information on polymorphic drug metabolism genes, and also identify genes differentially expressed in response to a drug. In fact, one gene chip, CYP2C6/CYP2C19, is already available for identifying potential poor drug metabolizers. On the other hand, this genomics-based technology might also help to understand the biological drug responses and to interpret therapeutic trials¹⁰⁸.

Comparing mRNA expression profiles can be used to explore which genes are up-regulated or down-regulated in osteoporosis treatment by comparing the expression profiles in tissue taken from affected and unaffected individuals. The potential difficulty with this approach is that small variations in the cellular constituents of the tissue might produce large fluctuations in mRNA and/or protein, giving rise to false positive (or negative) results. Another potential problem is that the logistical difficulties of dealing with data on thousands of gene products (which by definition may have no known function) are considerable. These problems can be avoided to some extent by simplifying the experimental design. For example, one approach is to use cultured human bone cells from a single individual and then to compare expression profiles after treatment with, say, bisphosphonates.

Apart from the logistical difficulties in sampling from bone and obtaining comparable bone tissue, study design will also be a major issue. As with genetic linkage studies, a major challenge for pharmacogenomics of osteoporosis lies in the design of meaningful studies for use of these technologies. In any mRNA-level study, a reasonable number of paired replicates must be performed and relevant time points examined. In practice, it may be possible to reduce this to a baseline and two different time points for this kind of experiment. However, even then, with an appropriate number of replicates, the number of samples to be processed and the logistics of multiple samples, at least in humans, remain daunting if not ethically impossible.

On the other hand, large epidemiological studies are required to identify associations between specific gene polymorphisms and predisposition to osteoporosis before these could be useful in clinical settings. At present, large-scale

SNP-based association studies in osteoporosis are feasibly prevented by limitations in genotyping resources and biostatistical models. Large-scale association studies involving SNPs will be more practical when high-throughput and affordable SNP scoring methods are available¹⁰⁹. The progress has, nevertheless, been impressive: to date, the Human Genome Project has provided more than two million SNPs as genetic markers¹¹⁰. Within the next few years, SNPs located every 3-50 kb will likely be characterized, it will be possible to perform genome-wide association studies to obtain information about major genes that contribute to the disease or pharmacological differences, as well as secondary, modifier, genes that also affect the disease. The recent development of a single mouthwash method for obtaining genomic DNA clinical studies¹¹¹ may be suitable for large community-based studies in which samples can be collected by the participants themselves.

The advance of genomic research gives rise to several ethical issues that need to be resolved. While information such as race and ethnicity have long been used in predicting therapeutic response, a growing number of critics view the use of this information as potentially prejudicial¹¹². Collecting and storing genetic information from individuals raise questions of privacy as well as security and ethical dilemmas, since the information also provides information about potentially non-censored relatives. Thus, guidelines need to be developed to protect the privacy and confidentiality of participants and their family members. A critical component of any such study will be to ensure that the ethical principle of beneficence is fulfilled. The analysis of DNA samples, including those from large population-based studies, in research is very important for understanding genetic influences of disease susceptibility, but the benefit must be weighed against risk to persons, including the potential for discrimination and invasion of privacy. Although these issues are difficult, it has been suggested that treating participants as limited partners in genetic research can provide a framework for addressing many of these concerns¹¹³.

In summary, data accumulated during the last three decades clearly indicate that genetic factors are a major determinant of bone mineral density, quantitative ultrasound of bone, and bone turnover. Many genetic factors appear to be involved in the determination of BMD as well as bone architecture in various skeletal sites. From the clinical as well as economic points of view, aggressive strategies to search for osteoporosis genes are warranted. With the progress of the Human Genome Project, a new era of post-genomics genotype – phenotype correlations in osteoporosis is heralded. The identification of relevant genes should enhance our understanding not just of disease mechanisms, but also explain why the clinical course of osteoporosis is so variable among individuals. Much more operational research is required to design studies capable of deciphering the complex interactions between individuals' genetic differences, predisposition to the disease, and drug-gene interactions; and the integration and interrogation of the vast data sets that

such studies will produce. While such research effort is undoubtedly complex, the ongoing development of molecular techniques in pharmacogenomics may allow not only individual prediction of drug efficacy and toxicity but also the development of innovative, more active and safer drugs. Genotyping individuals can help determine the influence of the polymorphisms on the pharmacokinetics of the drug on a number of enzymes and transporters that influence the processes of drug absorption and metabolism. Genotyping could also be used to stratify patients for phase III trials, to reduce the necessary sample size. Likewise, genotyping may become part of routine investigations to help clinicians tailor drug therapy effectively. Recent studies demonstrate the feasibility and the importance of these concepts^{108,114}.

The Human Genome Project, coupled with new molecular technologies and new statistical methods, will collectively enhance the search for osteoporosis genes and help translate the prediction of genetically complex osteoporosis into the realm of the possible.

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