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Genetic Profiling and Individualized Prognosis of Fracture

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ABSTRACT

Fragility fracture is a serious public health problem in the world. The risk of fracture is determined by genetic and nongenetic clinical risk factors. This study sought to quantify the contribution of genetic profiling to fracture prognosis. The study was built on the ongoing Dubbo Osteoporosis Epidemiology Study, in which fracture and risk factors of 858 men and 1358 women had been monitored continuously from 1989 and 2008. Fragility fracture was ascertained by radiologic reports. Bone mineral density at the femoral neck was measured by dual-energy X-ray absorptiometry (DXA). Fifty independent genes with allele frequencies ranging from 0.01 to 0.60 and relative risks (RRs) ranging from 1.01 to 3.0 were simulated. Three predictive models were fitted to the data in which fracture was a function of (1) clinical risk factors only, (2) genes only, and (3) clinical risk factors and 50 genes. The area under the curve (*AUC*) for model 1 was 0.77, which was lower than that of model II (*AUC* = 0.82). Adding genes into the clinical risk factors model (model 3) increased the *AUC* to 0.88 and improved the accuracy of fracture classification by 45%, with most (41%) improvement in specificity. In the presence of clinical risk factors, the number of genes required to achieve an *AUC* of 0.85 was around 25. These results suggest that genetic profiling could enhance the predictive accuracy of fracture prognosis and help to identify high-risk individuals for appropriate management of osteoporosis or intervention. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: OSTEOPOROSIS; FRACTURE; GENETIC PROFILING; RECLASSIFICATION ANALYSIS; INDIVIDUALIZED PROGNOSIS

Introduction

Fracture is an important public health problem because, among others things, it is common in the general population and is associated with increased risk of death. Approximately 44% of women and 25% of men aged 50 years will sustain a fracture during their remaining lifetime.[1] Individuals with a preexisting fracture are at increased risk of subsequent fracture[2] and death.[3] Pharmacologic treatment of individuals with a preexisting fracture can reduce further fracture[4] and mortality risk.[5]

Therefore, a major priority in osteoporosis research at present is to develop prognostic models for identifying individuals who have high risk of fracture. Using established clinical risk factors, a number of prognostic models have been developed and implemented recently.[6–8] The predictive accuracy of these models has been less than perfect, with the area under the receiver operating characteristic curve (*AUC*) ranging between 0.70 and 0.80.[7,8] Most prognostic models have low sensitivity and high specificity. Thus there is room for further improvement of prognostic accuracy of the current models.

Fracture segregates within families, but the segregation does not follow the Mendelian law. Women whose mothers had sustained a hip fracture have a twofold increase in risk of hip fracture compared with controls,[9] but the penetrance is not complete. Indeed, approximately 25% to 35% of the variance in the liability to fracture was attributable to genetic factors.[10,11] Moreover, genetic factors also account for a large proportion of variance in risk factors for fracture, such as bone mineral density (BMD),[12] bone loss,[13] quantitative ultrasound,[14] and bone turnover markers.[15] Thus genetic factors may be useful in the prognosis of fracture. However, studies in the fields of diabetes[16] and cancer[17] suggested that genetic profiling contributed minimally to the prognosis of these diseases.

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Although it is clear that genetic factors play a role in the pathogenesis of fracture liability, it is less clear which specific genes may be associated with fracture risk. Indeed, several studies during the past two decades have suggested a number of candidate genes for osteoporosis,[18] but the lack of independent replication has been a hindrance to progress of the field. In recent years, genome-wide association studies have been conducted, and a number of novel genes have been found to be associated with fracture risk. A meta-analysis of 150 candidate genes found only 4 genes that were significantly associated with fracture.[19] Two common features of these genes are that their allelic frequency in the general population is highly variable (ranging from 1% to 61%) and that the effect size is very small. In the presence of such small effect size, it can be anticipated that the contribution of any single gene or single-nucleotide polymorphism (SNP) to fracture prognosis is minimal.

We hypothesize that a genetic profiling or combination of several genes can improve the prognostic accuracy of fracture. This study therefore was undertaken to test the hypothesis by addressing the following two specific questions: (1) What is the contribution of a single gene to fracture risk prediction? and (2) What degree of fracture risk can be predicted by simultaneous testing of multiple genetic variants (ie, genetic profiling)?

Study Design and Methods

Study setting and participants

This study was built on the ongoing prospective Dubbo Osteoporosis Epidemiology Study (DOES), in which details of protocol and study design have been described previously.[20–23] Briefly, in 1989, all men and women aged 60 years or above (as of 1989) living in Dubbo, a city of approximately 32,000 people 400 km northwest of Sydney (Australia), were invited to participate in an epidemiologic study. At that time, the population consisted of 1581 men and 2095 women aged 60 years or older, of whom 98.6% were white and 1.4% were indigenous aboriginal. These individuals were all invited to participate in DOES. This study was approved by the St Vincent's Campus Research Ethics Committee, and written informed consent was obtained from each participant.

Dubbo had been selected for the study because the age and gender distribution of its population closely resembled the Australian population,[22] and it is relatively isolated in terms of medical care so that virtually complete ascertainment of all fractures in the target population is possible.

Assessment of clinical risk factors

At baseline, bone mineral density (BMD, g/cm^2) was measured at the lumbar spine or femoral neck (FN) by dual-energy X-ray absorptiometry (DXA) initially using a DPX densitometer (GE-Lunar Corp., Madison, WI, USA). The radiation dose with this method is less than 0.1 μGy. The coefficients of reliability of BMD in our institution in normal subjects are 0.96 and 0.98 at the proximal femur and lumbar spine, respectively.[24] In this analysis, baseline femoral neck BMD, not lumbar spine BMD, was used because the former is minimally affected by degenerative changes that may artificially elevate BMD.

Individuals also were interviewed by a nurse coordinator, who administered a structured questionnaire to obtain data including age, any history of fracture after the age of 50 years, and history of falls in the preceding 12 months. Anthropometric variables (ie, height and weight) were measured at baseline.

Ascertainment of fracture

The incidence of fracture was ascertained during the period of follow-up, which had taken place between 1989 and 2008. Low-trauma and nonpathologic fractures were considered the primary outcome of this study. Fractures occurring during the study period were identified for residents of the Dubbo local government area through radiologists' reports from the two centers providing X-ray services, as described previously.[20,21] Fractures were included only if the report of fracture was definite and, on interview, had occurred with low trauma (eg, fall from standing height or less). Fractures clearly owing to major trauma (eg, motor vehicle accidents), those owing to underlying diseases (eg, cancer or bone-related diseases), or those of digit, skull, or cervical spine were excluded from the analysis. This study did not include morphometric vertebral fractures.

Simulation of genotypes

With the number of fractures and nonfractures that have been observed during the study period, we simulated 50 genes with the following assumptions: (1) the *allele frequency* of the genes ranged between 0.01 and 0.60, (2) the *relative risk* ranged between 1.1 and 3.0, and (3) the *genes were independent from each other*. We specified the parameters of simulation such that genes with low allele frequency have greater relative risk than genes with high allele frequency (Supplemental Table S1). Since we know the number of fractures and nonfractures in the study, the simulation of genotypes was based mainly on two parameters: allele frequency and relative risk. Let the incidence of fracture be p; the allele frequencies $f = \{f_1, f_2, \ldots, f_M\}$, where f_i corresponds to the ith gene; and the relative risk $RR = \{RR_1, RR_2, \ldots, RR_M\}$ of the heterozygous genotype corresponding to each gene. Assuming that each single gene has two alleles and that all genotypes were in Hardy-Weinberg equilibrium, the number of fractures and nonfractures for each genotype in the population can be determined from the parameter f. For each gene, a vector of length N was created containing the genotypes corresponding to N individuals, with proportion of each genotype as specified by the parameter f. Each subject was assigned a genotype by randomly sampling (without replacement) this vector.

The relative risk of fracture for each risk allele was simulated based on recent results of candidate gene association and genome-wide association studies (GWAS). For example, the minor genotype of a variant located within the Sp1 binding of the *COL1A1* gene was consistently shown to be associated with fracture in a dominant manner, such that the TT genotype (6.3% in the general population) was associated with a 4.21-fold increased risk of hip fracture compared with GG and GT genotypes.[25] Moreover, the minor genotype GG of the variant in exon 15 of the *LRP5* gene (11% in the general population) was associated with a 1.67-fold increased risk of fracture compared with other genotypes.[26,27] Recent GWAS results also suggested

that virtually all statistically significant SNPs conferred a relative risk of fracture of between 1.1 and 1.4.[19]

Data analysis

Our primary aim was to assess the relative contribution of genetic profiling to the prognosis of fracture. To address that aim, we considered three predictive models as follows: (1) Model 1 included only clinical risk factors that have been shown to be associated with fracture risk. These risk factors included sex, femoral neck BMD, history of prior fracture, falls during the past 12 months, and age. (2) Model 2 included only genetic profiling. The genetic profiling was quantified by two ways as follows: In the first approach, for each individual, each gene was coded as 1 (for presence of the high-risk allele) or 0 (for absence of the high-risk allele), and the summation of all 50 scores was termed the *genetic risk score*. In this second approach, we included all 50 genes as 50 independent risk factors in the predictive model. (3) Model 3 included both clinical risk factors and genetic profiling.

The predictive accuracy of a model was assessed by the area under the receiver operating characteristic curve (*AUC*),[28–31] which is the probability that given two randomly drawn individuals, the individual who will sustain a fracture first had a lower probability of nonfracture. In recent years, it has been realized that the *AUC* is too insensitive to change[32] and that it may not be the most appropriate method for assessing the contribution of genetic markers. Therefore, in order to assess the incremental prognostic value attributable to genetic profiling, a reclassification analysis[33] was performed. In this reclassification analysis, the probability of fracture was estimated for each individual by each model and then classified into three risk groups: less than 10%, between 10% and 20%, and more than 20%. The proportion of individuals who would be reclassified into the three risk groups for the model with genetic profiling and the model without the genetic profiling was calculated. Thus, if genetic profiling is useful for fracture prognosis, the probability of fracture estimated by the model with genetic factors would be increased for the fracture group and decreased for the nonfracture group. We quantified this prognostic improvement by computing two indexes: the *net reclassification improvement* (NRI) and the *integrated discrimination improvement* (IDI). The NRI was computed as follows: NRI = Pr(up | fracture) – Pr(down | fracture) – Pr(up | nonfracture) + Pr(down | nonfracture), where Pr denotes probability. The IDI was computed as $IDI = P_{1,fx} - P_{1,\text{nonfracture}} - P_{0,\text{fracture}} + P_{0,\text{nonfracture}}$, where $P_{1,fx}$ is the average predicted probability of fracture in the fracture group for the model with genetic factors, $P_{1,\text{nonfracture}}$ is the average predicted probability of fracture in the nonfracture group for the model with genetic factors, $P_{0,\text{fracture}}$ is the average predicted probability of fracture in the fracture group for the model without genetic factors, and $P_{0,\text{nonfracture}}$ is the average predicted probability of fracture in the nonfracture group for the model without genetic factors. The IDI also can be interpreted as the proportion of variance explained by genetic factors. All analyses were performed using the R language on the Windows platform (Open Source, R Development Core Team, Vienna, Austria)[34] with the Design and Hmisc packages (Frank E Harrell Jr et al., Vanderbilt University, Nashville, TN, USA).

Results

There were 858 men and 1358 women whose full data were available for analysis. During the follow-up period (1989 and 2008), 17% ($n = 149$) of the men and 31% ($n = 426$) of the women had sustained a fragility fracture. As in previous studies, individuals with a fracture had, on average, lower BMD than those without a fracture.[35–38] Moreover, individuals with a fracture were older, more likely to have a preexisting fracture, and more likely to have had at least one fall during the 12 months prior to the fracture event.

The distribution of simulated genetic risk scores had a median genetic risk score of 13 (range 4 to 25; Fig. 1). Approximately 10% of individuals had fewer than 10 risk genotypes; another 10% had more than 16 risk genotypes. Compared with those with fewer than 10 risk genotypes, those with 10 to 16 risk genotypes had their odds of fracture increased by 5.47 [95% confidence interval (CI) 3.03–9.90]. Those with more than 16 risk genotypes had the highest risk of fracture [odds ratio (OR) = 43.6; 95% CI 22.8–83.2].

The *AUC* of the model with only genetic risk score was 0.78, which was not significantly different from that of the model with clinical risk factors alone (ie, sex, age, femoral neck BMD, prior fracture, and fall). However, the model in which 50 individual genes were analyzed had an *AUC* of 0.82, significantly higher than either the model with a single genetic risk score or the model with clinical risk factors. When the genetic risk score was added to the clinical risk factors, the *AUC* was increased to 0.85 ($p < .0001$). When the individual 50 genes were added to the model with clinical risk factors, the *AUC* was increased to 0.88, which was the "best" model for predicting fracture risk (Table 1).

The effect of sequentially adding each gene into the predictive model was assessed by the incremental cumulative *AUC* values. For example, a model with three clinical risk factors (ie, age, prior fracture, and fall) yielded an *AUC* of 0.732; add gene 1 (frequency = 0.6; RR = 1.10) increased the *AUC* to 0.733; add gene 2 (frequency = 0.60; RR = 1.11) on top of gene 1 increased the *AUC* to 0.734. The *AUC* was increased to 0.80 when the model had 25 genes. However, in the presence of BMD, the number of genes required to increased the *AUC* to 0.80 was 20 (Fig. 2).

Reclassification analysis (Table 2) showed that compared with the clinical risk factors model, the model with an additional

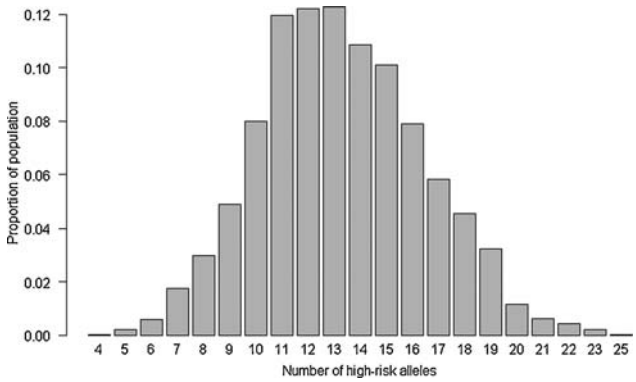

Fig. 1. Distribution of the number of simulated "high-risk" alleles in the Dubbo population.

Table 1. Area Under the Receiver Operating Characteristic Curve for 3 Models

Model	*AUC*	*AUC* Improvement over the model with CRF only
1. Sex + age + BMD + prior fracture + fall	0.77	
2a. Genetic risk score (GRS)	0.78	0.01
2b: Gene1 + Gene2 + Gene3 + . . . + Gene50	0.82	0.05
3a: Sex + age + BMD + prior fracture + fall + GRS	0.85	0.08
3b: Sex + age + BMD + prior fracture + fall + 50 genes	0.88	0.11

genetic risk score reclassified nonfracture individuals into lower risk groups by 45% but reclassified 12% into higher risk groups; thus there was a gain of 33%. For the fracture group, the model with genetic risk score reclassified upward 14% but downward by 11%, a gain of 3%. In total, there was a net gain of 33 + 3 = 36% (Supplemental Table S2). However, the model with individual 50 genes and clinical risk factors yielded a net gain of 46% over and above the model with only clinical risk factors (Supplemental Table S3).

The inclusion of either genetic risk score or 50 individual genes increased the predicted probability of fracture in the fracture group and decreased the predicted probability of nonfracture in the nonfracture group. However, while the genetic risk score explained approximately 14% of the fracture risk variance, the individual 50 genes explained approximately 20% of the fracture risk variance (Table 3).

Discussion

The assessment of fracture risk is currently moving toward an absolute-risk approach, in which an individual's risk of fracture is estimated from the individual risk profile. At present, the risk profile is largely based on clinical risk factors such as history of prior fracture, history of a fall, bone mineral density, body weight, and concomitant diseases.[6–8] Although these clinical risk factors are known to be associated with fracture risk, their ability to discriminate fracture from nonfracture cases is modest, with the *AUC* value ranging from 0.70 to 0.80.[7,8] Therefore, there is room for further improvement of fracture prognosis, and genetic profiling is potentially an important contributor to the enhancement of fracture risk assessment.

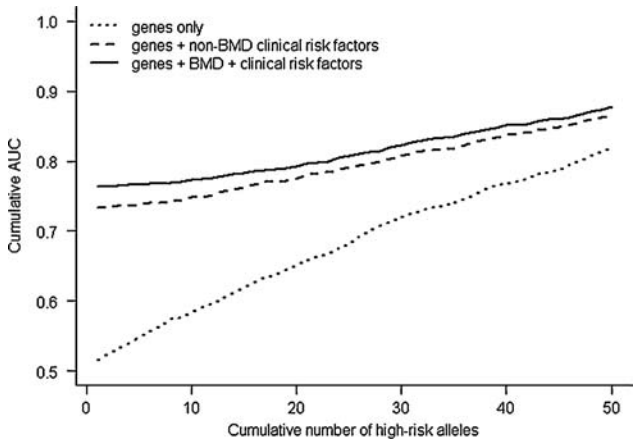

Fig. 2. Cumulative *AUC* as a function of number of genes only *(dotted line)*, with genes + non-BMD clinical risk factors *(dashed line)*, and with genes + BMD + clinical risk factors *(solid line)*.

It is well established that the risk of fracture is partially determined by genes, but it is unclear whether genetic risk factors can improve the prognosis of fracture over and above of that provided by clinical risk factors. In this study, we have shown that the contribution of any single gene to the prognosis of fracture is very small. Indeed, even for a gene conferring a relative risk of 3, the *AUC* attributable to this gene is barely 0.51 (in the absence of clinical risk factors). Even with five genes, each conferring a relative risk of between 2 and 3, the *AUC* is still 0.60, which is not useful for predicting fracture. This finding suggests that the contribution of any single gene to fracture prognosis, no matter how large, the effect size likely is limited and would not be useful, particularly in a clinical setting. However, the integration of genetic profiling, in the form of either a genetic risk score or individual genes, into the current prognostic models could improve the predictive accuracy of fracture risk significantly for an individual.

It is now clear that the risk of fracture is determined by many genes, each with a *small* effect size. This is perhaps not surprising given the number of complex phenotypes and the number of regulatory proteins involved in calcium, collagen, bone metabolism, bone strength, and bone size. This analysis suggested that a profile of up to 25 genes (each with a relative risk of 1.1 to 1.35 and gene frequency ranging from 0.25 to 0.60) in the presence of clinical risk factors—with or without BMD—is required to achieve an *AUC* of 0.80, indicative of clinical usefulness. Until now, very few genes have been implicated in

Table 2. Reclassification Analysis: The Effect of Adding Genetic Profile into the Basic (Clinical Risk Factor) Model

Comparison of model	Percent of reclassification compared with model with clinical risk factors only				
	No-fracture group		Fracture group		
	Down	Up	Down	Up	NRI
Model 3a	0.45	0.12	0.11	0.14	0.36
Model 3b	0.52	0.11	0.11	0.16	0.46

NRI = net reclassification improvement.

Model 3a included sex, age, BMD, prior fracture, fall, and genetic risk score.

Model 3b included sex, age, BMD, prior fracture, fall, and 50 genes.

Table 3. Average Predicted Probability of Fracture by Models With and Without Genetic Profiling for the Fracture and No-fracture Groups

	Model without genes		Model with genes		
Comparison of models	Fracture group	No-fracture group	Fracture group	No-fracture group	IDI
Model 2 versus 3a	0.414	0.198	0.518	0.163	0.139
Model 2 versus 3b	0.414	0.198	0.562	0.148	0.198

IDI = integrated discrimination improvement.
Model 2 included only genetic risk score.
Model 3a included sex, age, BMD, prior fracture, fall, and genetic risk score.
Model 3b included sex, age, BMD, prior fracture, fall, and 50 genes.

the determination of fracture risk. A recent meta-analysis of 150 SNPs found that only 5 SNPs from four genes were consistently associated with fracture risk, with relative risk ranging from 1.1 to 1.4.[19] Thus, given the ongoing progress of finding new genes for osteoporosis, the prospect of using genetic profiles in the prognosis of fracture is a real possibility.

The aim of individualized prognosis is to provide an accurate and reliable prognosis of fracture for an individual and to help improve management of the individual's predisposition to fracture. Each individual is a unique case because there exists no "average individual" in the population. The uniqueness of an individual can be defined in terms of the individual's environmental and genetic profile. Thus the knowledge of genetics, in combination of clinical risk factors, can shift our current risk-stratification (ie, "one-size-fits-all") approaches to a more individualized evaluation and treatment of osteoporosis.

These findings should be interpreted within a number of strengths and weaknesses. The strength of the study is that the risk factors and fracture data were ascertained from a well-characterized cohort with long duration of follow-up. The genes were simulated to closely resemble the real-world situation, in which multiple genes affect an individual's fracture risk with a small to modest effect size. As such, although the study was partially simulated, it was really an empirical study. The model we considered here was based on the assumption that the effects of all 50 genes were totally independent. Although most past findings have suggested that the effects of genes were independent, there is no reason to think that genes exert their effects on fracture risk independently. Considering the complex phenotypes of osteoporosis, it would be expected that the effect of a certain gene in part depends on other genes or environments (ie, gene and gene-environment interactions). However, identification of these interactions is quite a challenge because of current linear statistical genetic methods used for analyzing and detecting gene-phenotype associations in human populations are not sensitive enough to detect nonlinear interacting effects owing to the combinatorial complexity of gene-gene and gene-environment interactions.

The utility of genes as a prognostic tool for predicting common chronic diseases has been challenged.[41] Empirically, it has been shown that the incorporation of genes did not result in an appreciable increase in the accuracy of the prognosis of type 2 diabetes[42] and cardiovascular diseases,[39,40] which suggests that common genetic variants have a minimal effect on the prognosis of these diseases. However, the mentioned work assessed the incremental prognostic value based on the *AUC* rather than the reclassification analysis as in our work. However, *AUC* is insensitive to the addition of new and statistically significant predictors, and the reclassification analysis provides a better quantification of prognostic improvement.

In summary, we have demonstrated in this study that genetic profiling could improve the prognosis of fracture risk significantly and help to identify high-risk individuals for appropriate risk management or intervention.

Disclosures

All the authors state that they have no conflicts of interest.

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