COMMENTARIES

Genetics of Osteoporosis: From Population Association to Individualized Prognosis of Fracture

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Osteoporosis and its consequence of fracture, as other chronic diseases, impose a significant demand on medical care and health services. This is true because fracture is associated with a series of adverse outcomes, such as increased risk of morbidity and disability (1), excess risk of mortality (2;3), increased loss of productivity, and ultimately incurs a significant health care cost (4). One of the major priorities in osteoporosis research is the development of prognostic models for identifying individuals at high risk of fracture for early intervention and management (5). Such prognostic models have been developed by making use of traditional clinical risk factors such as advancing age, bone mineral density (BMD), prior fracture, and comorbidities (6-8). Because fracture has a genetic component that is independent of traditional clinical risk factors, it is logical to assume that the prognosis of fracture could be improved by including genetic factors. Some of the latest developments in the field of osteoporosis genetics have suggested that such clinico-genetic prognostic models could be realized in the near future (9).

From Frustration to Promise

Through several twin and family studies, it is now clear that deviations from health attributable to osteoporosis segregate within families; however, the segregation does not follow the genetic laws seen in single-gene Mendelian disorders (10). Women whose mothers have experienced a hip fracture exhibit a two-fold increase in risk of hip fracture compared with controls (11), but the penetrance is not complete. Indeed, approximately 25-35% of the variance in the liability to fracture is attributable to genetic factors (12;13). Moreover, genetic factors also account for a large proportion of variance in risk factors for fracture such as BMD (14), bone loss (15), quantitative ultrasound (16), and bone turnover markers (17).

The recognition that various bone-related traits are largely determined by genetic factors has led to an intensive search for specific genes either linked with these traits or with fracture risk. The search for the link between osteoporotic fracture and random strands of DNA has gone through a period of frustration to a period of promise. Gene-search studies have been based on the two major approaches of genome-wide linkage analysis and candidate gene association analysis (18). 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, several gene polymorphisms (including vitamin D receptor, collagen type Iα1, osteocalcin, IL-1 receptor antagonist, calcium-sensing receptor, α2HS glycoprotein, osteopontin, osteonectin, estrogen receptor α, interleukin-6, calcitonin receptor, collagen type Iα2, parathyroid hormone, and transforming growth factor α1 polymorphisms) have been proposed (19). However, the decade in which candidate gene association studies have blossomed has also been accompanied by increasing frustration with conflicting findings and a lack of independent replication, mainly due to, among other reasons, a lack of statistical power (20) and to false positives (21).

Genome-wide linkage analysis has also been utilized in the search for genes that are linked to osteoporosis, and have yielded significant results. By using linkage analysis of data from a family with osteoporosis-pseudoglioma syndrome (OPPG), a disorder characterized by severely low bone mass and eye abnormalities, investigators were able to localize the OPPG locus to chromosomal region 11q12-13 (22). 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 the 30cM region of the same locus (23). 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 OPPG and high bone mass (24-26). The finding that the LRP5 gene is linked to HBM was subsequently confirmed in a family study that included individuals with exceptionally high BMD but who were otherwise phenotypically normal (25). This study showed that a missense mutation (G171V) was found in individuals with high BMD (26). A recent family study further identified six novel mutations in the LRP5 gene among 13 confirmed polymorphisms that were associated with different conditions characterized by increased BMD (27). The conditions included endosteal hyperostosis, van Buchem disease, autosomal dominant osteosclerosis, and osteopetrosis type I. Perhaps it is reasonable to state that the discovery of the LRP5 gene has opened up a new chapter of research in the genetics of osteoporosis.

**Evaluation of the Association Between the LRP5 Gene, BMD and Fracture**

However, the above associations were based on selected samples, and not on the general population. Since the identification of the LRP5 gene, there have been several population-based studies on the association between LRP5 polymorphisms and normal variation in BMD, again with some inconsistent findings. In the presence of such inconsistent results, a meta-analysis seems appropriate. van Meurs et al. (9) have just conducted a meta-analysis of 37,534 individuals from 18 study populations in Europe and North America, and found that 2 common variants (Val667Met and Ala1330Val) within the LRP5 gene were associated with BMD and fracture risk. For example, they found that carriers of the Val667Met variant’s MetMet genotype were associated with 20 mg/cm² lower lumbar spine BMD (p = 3.3 x 10⁻⁸) and 11 mg/cm² lower femoral neck BMD (p = 3.8 x 10⁻⁵), compared to those with MetVal and ValVal genotypes. The ValVal genotype within the Ala1330Val variant (rs3736228) was associated with 16 mg/cm² lower lumbar spine BMD (p = 3.4 x 10⁻⁵) and 10 mg/cm² lower femoral neck BMD (p = 9.9 x 10⁻⁷) compared to that in AlaVal and AlaAla genotypes.

These results from the analysis by van Meurs et al. are quite comparable to a recent genomewide association study in which Richards et al. (28) reported that the Ala1330Val variant was associated with BMD with an effect size of 0.13 standard deviation (SD) and a p-value of 6.3 x 10⁻¹². In a summary-based meta-analysis (29), using the Bayesian approach, it was shown that the probability of an effect size (AlaAla
to the false positive rate
association size
observed power (s
value (equivalent to 1 minus specificity), the
analogous parameters: the observed p
association can also be evaluated by three
(PPV), the reliability of a statistical
test, where on
Just as
alternatively, what is the probability of a
probability that there is a true association, or
finding or the
association given a statistically significant
false positive rate probability (FPRP) (34).

Of the three parameters for evaluating FPRP, the prior probability is the most
difficult parameter on which to put weight. This probability is dependent on the number
of gene variants that affect fracture susceptibility, which is unknown. Indeed, we
do not know how many genes are involved in the regulation of, or are relevant to, the
underlying susceptibility to osteoporotic fracture. However, we do know that in the
human genome, there are about 3 billion base pairs (35), and that on average, more
than 90% of the differences between any two individuals is due to common variants
where both alleles are present in at least 1% of the population (36). Therefore, it has been
hypothesized that the susceptibility to common diseases such as osteoporosis is
caused by a few common genetic variants with low effect size (i.e., the “common gene
– common variant” hypothesis) (37). Under this hypothesis, it has been estimated that
the number of genetic variants that are
associated with a common disease is about
100 or less (38). It has also been estimated that the number of common variants in the
human population is about 10 million (39). Therefore, it may be reasonable to assume
that the probability that a randomly selected common variant is associated with the risk
of fracture is 1/100,000 or 0.000001. If there is a priori biologic justification and prior
evidence of association with BMD, such as
in the case of the LRP5 gene, this
probability may be around 0.001.

Setting the prior probability of association at
0.001 and 0.000001 to correspond to the
levels that would be expected for a
candidate gene and for a random SNP in a
genomewide association analysis,
Table 1. Evaluation of false positive probabilities in the reported associations between LRP5 variants and fracture risk. *Data from Richards et al. (28). OR: odds ratio; FPRP: false positive report probability.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Reported OR</th>
<th>Prior probability = 0.000001</th>
<th>Prior probability = 0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR=1.1</td>
<td>OR=1.2</td>
</tr>
<tr>
<td>Val667Met</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.09</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>(0.93-1.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1.17</td>
<td>0.965</td>
<td>0.837</td>
</tr>
<tr>
<td></td>
<td>(1.06-1.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala1330Val</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.07</td>
<td>0.995</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>(0.97-1.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1.06</td>
<td>0.993</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>(1.00-1.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile1062Val</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.98</td>
<td>0.999</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>(0.90-1.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1.01</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>(0.94-1.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala1330Val</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men +</td>
<td>1.30</td>
<td>0.982</td>
<td>0.864</td>
</tr>
<tr>
<td>women</td>
<td>(1.09-1.52)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

respectively, the reported association between LRP5 gene variants and fracture was evaluated, and results are shown in Table 1. As can be seen from the table, all reported associations between the LRP5 gene variants and fracture had a FPRP greater than 0.20, which means that none of these associations are noteworthy.

Nevertheless, the observed magnitude of association between fracture risk and LRP5 gene variants re-affirms the increasingly prevalent view that the susceptibility to fracture is determined by multiple genotypes, with each conferring a modest elevated risk (odds ratios ranging from 1.1 to 2.0). Assuming that this is a real scenario, how could genes help the prognosis of fracture for an individual?

**Genetics and Individualized Prognosis**

The assessment of fracture risk has until now been based on the measurement of BMD and a history of prior fracture. Although low BMD (e.g., osteoporosis) is the best predictor of fracture risk, it can not account for all fractures in the general population. Even at the lowest BMD range, only some individuals will sustain a fracture; on the other hand, a high BMD does not confer total protection against a fracture. Indeed, in individuals aged 60+ years, 55% and 74% of fracture cases occurred in non-osteoporotic women and men, respectively (32). As a result, treatment of individuals with a BMD-based threshold (e.g., osteoporosis) can reduce only a modest number of fractures in the general population. Therefore, important changes in thinking are needed for that majority of individuals whose BMD measurements are at or near, on both sides, the current threshold of osteoporosis.

Recently, we have developed a number of prognostic models, in which an individual’s multiple risk factors are simultaneously considered in a multivariable model and represented by a nomogram (7;40). An advantage of this nomogram-based approach is that it treats all continuous risk factors in their original units of measurement, and as a result, it obviates the need for grouping individuals by some arbitrary thresholds (such as osteoporosis vs. non-osteoporosis) that is inefficient and has poor predictive power. The use of continuous measurements and multiple risk factors increases the uniqueness of an individual and allows the risk of fracture to
be individualized. Thus, the nomogram-based model recognizes the fact that there are different ways two individuals can reach the same risk level.

Individualized prognosis of fracture is about imparting information of fracture risk to an individual, and each individual is a unique case, because no “average individual” exists in the population. As more risk factors are considered, the greater the likelihood of defining the uniqueness of an individual’s profile. One way to increase the uniqueness or individuality of prognosis is to combine genotypes and clinical risk factors. For example, with 10 gene variants (each with 2 alleles) there are 59,049 combinations of genotypes, and when these combinations are considered in relation to other risk factors such as age, BMD, and history of fracture, it is possible to individualize the prognosis of fracture for any particular individual in the general population.

However, in the presence of modest association between any gene variant and fracture risk, it is important to ask whether gene variants can contribute to the individualized prognosis of fracture risk. Experience in cancer research (41) suggests that a single genetic variant may not be clinically useful since it is not sufficient to improve risk prediction in an individual. However, as more gene variants are identified, it is theoretically possible to combine them into a genetic risk profile for an individual, and together with traditional clinical risk factors, the profile could be sufficient to provide a reasonably accurate individualized prognosis that can guide clinical decisions.

Consider a population among whom the 10-year risk of osteoporotic fracture is about 25%. Suppose that there are 5 unlinked (independent) genetic variants that are known to be associated with fracture risk with the following relative risks: 1.1, 1.2, 1.3, 1.5 and 5.0. Suppose that the genetic variants are present in 20%, 15%, 10%, 5%, and 1% of the population. Furthermore, suppose that each SD decrease in BMD and 5-year advancing age are associated with a 2-fold and 1.5-fold increase in fracture risk, respectively. It can be shown by simulation that the number of fracture cases that is attributable to the 5 gene variants is ~17%. This is so because only 8% of the population carry two or more risk genotypes, and none of the hypothetical population carries 5 risk genotypes (Table 2).

<table>
<thead>
<tr>
<th>Number of risk genotypes</th>
<th>Percent of population exposed to gene variants</th>
<th>Odds ratio of fracture and 95% confidence interval</th>
<th>Number needed for genetic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>57.7</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>34.4</td>
<td>1.3 (1.2-1.5)</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>7.2</td>
<td>2.0 (1.7-2.4)</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>2.2 (1.3-3.7)</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>0.03</td>
<td>7.1 (0.6-78)</td>
<td>3</td>
</tr>
</tbody>
</table>

*Table 2. Number of risk genotypes, percent of population with risk genotypes, and odds ratio of fracture.*

When the 5 gene variants are considered in a logistic regression model, the area under the receiver operating characteristic curve (AuROC) is only 0.56 – not a useful discrimination. However, when the variants are combined with age and BMD, the AuROC is increased to 0.75, which is clinically useful (Figure 1).

The effect of genes can also be viewed from another angle. The number needed for a genetic effect is the number of individuals with a genotype among whom one event can be expected to occur as the result of a genetic effect (42). This index for the combination of gene variants in the simulated population is shown in Table 2. Thus, individuals carrying 2 risk genotypes (about 7% of the general population) have an odds of fracture that is increased by 2-fold compared to those who do not carry any risk genotype, but the number needed for a genetic effect is only 8, which suggests that 1 out of 8 individuals with the genetic profile
will sustain a fracture as the result of a genetic effect. Considering the effect of genotypes in this way helps clarify the magnitude of genetic influence on fracture risk at the population level.

It should be noted that the above consideration is based on the assumption of no interaction effects between genes, and no interaction effects between genes and BMD or age. In reality, interactions between genes and environmental factors could exist (43), and in this situation, the incorporation of genotypes could increase the specificity of fracture prognosis.

**Summary and Conclusion**

Arguably, osteoporotic fracture has an infinite set of causes. Fracture is caused by (among other causes) low BMD, which is caused by (among other causes) high bone turnover, which is caused by (among other causes) hormonal imbalance, which is caused by (among other causes) genetic factors or DNA damage, and so on.
Moreover, an individual’s current risk level is partially a function of the individual’s previous levels. Therefore, it can be hypothesized that osteoporotic fracture occurs as a consequence of an interaction between the “initial” condition coded in the genes, and exposure to hormonal and environmental factors indexed by time and space (44). Thus, the risk of fracture for an individual at a particular time in a particular environment is influenced by the phenotype produced by a prior genotype-environment interaction. Therefore, a prognosis of fracture should ideally take into account the full knowledge of an individual’s genetic profile and his/her clinico-environmental exposure.

We do not know exactly how many genes are involved in the regulation of fracture susceptibility. We also do not know their mode of inheritance or their frequency in the general population. In fact, with current methodology, it is unlikely that we will completely understand the causes of fracture, and why some individuals fracture and others do not. However, it is possible to find risk factors that account for a substantial number of cases and that are amenable to intervention. Newly identified genetic variants in combination with clinical risk factors can help improve the accuracy of prognosis of fracture for an individual, and segregate individuals at higher risk of fracture from those with lower risk and hence lead to better management of the burden of osteoporosis in the general community.

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References


