

## Genetic Determination of Colles' Fracture and Differential Bone Mass in Women With and Without Colles' Fracture

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### ABSTRACT

Osteoporotic fractures (OFs) are a major public health problem. Direct evidence of the importance and, particularly, the magnitude of genetic determination of OF per se is essentially nonexistent. Colles' fractures (CFs) are a common type of OF. In a metropolitan white female population in the midwestern United States, we found significant genetic determination of CF. The prevalence ( $K$ ) of CF is, respectively, 11.8% ( $\pm$ SE 0.7%) in 2471 proband women aged 65.55 years (0.21), 4.4% (0.3%) in 3803 sisters of the probands, and 14.6% (0.7%) in their mothers. The recurrence risk ( $K_0$ ), the probability that a woman will suffer CF if her mother has suffered CF is 0.155 (0.017). The recurrence risk ( $K_s$ ), the probability that a sister of a proband woman will suffer CF given that her proband sister has suffered CF is 0.084 (0.012). The relative risk  $\lambda$  (the ratio of the recurrence risk to  $K$ ), which measures the degree of genetic determination of complex diseases such as CF, is 1.312 (0.145;  $\lambda_0$ ) for a woman with an affected mother and 1.885 (0.276;  $\lambda_s$ ) for a woman with an affected sister. A  $\lambda$ -value significantly greater than 1.0 indicates genetic determination of CF. The terms  $\lambda_0$  and  $\lambda_s$  are related to the genetic variances of CF. These parameters translate into a significant and moderately high heritability (0.254 [0.118]) for CF. These parameters were estimated by a maximum likelihood method that we developed, which provides a general tool for characterizing genetic determination of complex diseases. In addition, we found that women without CF had significantly higher bone mass (adjusted for important covariates such as age, weight, etc.) than women with CF. (J Bone Miner Res 2000;15:1243–1252)

**Key words:** Colles' fracture, heritability, osteoporotic fracture, genetic determination, relative risk, recurrence risk

### INTRODUCTION

More than 1.3 million osteoporotic fractures (OFs) occur each year, with an estimated direct cost of \$13.8 billion<sup>(1)</sup> in the United States alone. One central objective of bone biology is the investigation into all the important intrinsic and extrinsic factors that underlie OFs, with the ultimate goal to intervene effectively and reduce the risk and incidence of OFs. The majority of the studies<sup>(2–6)</sup> have concentrated on extrinsic and nongenetic environmental factors. Extensive studies<sup>(7–12)</sup> have been conducted to

define the relative importance of genetic factors in determining some risk factors underlying OFs. These studies have unambiguously revealed that ~50–80% of bone mineral density (BMD), a major risk factor for OF,<sup>(13–15)</sup> is under genetic control. The importance of genetic determination of other identified major risk factors (such as bone loss rates and bone size) also is suggested.<sup>(16–20)</sup> However, direct evidence of the genetic determination of OFs is essentially nonexistent. Particularly, the magnitude of the genetic determination of OFs per se is unknown.

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TABLE 1. CF STATUS FOR THE PROBANDS AND THEIR SISTERS AND MOTHERS

No. of probands	No. of sisters of the probands			No. of mothers of the probands			Total
	Affected	Unaffected	Total	Affected	Unaffected	Total	
293 (affected)	32	341	373	56	237	293	959
2178 (unaffected)	137	3293	3430	304	1874	2178	7786
2471 (total)	169	3634	3803	360	2111	2471	8745

Extensive molecular genetic studies<sup>(21–26)</sup> have been launched to search for genes underlying BMD variation. The results so far have been inconsistent, and consensus needs to be developed by further studies and by analyses of previous extensive results. Molecular genetic studies of other major risk factors (such as bone loss and bone size) have been scarce, even if important genetic determination has been revealed for them.<sup>(16–20)</sup> Direct molecular genetic studies of the OF per se are even more rare. Particularly, systematic, whole genome searches for genes important for OFs per se essentially do not exist. However, searches for genes underlying the risk of OFs per se are essential (see Discussion). OF occurs at different skeletal sites for which the pathogenesis and risk factors (including their underlying genetic loci if any) and/or their relative importance may not all be the same.<sup>(27,28)</sup> Almost all fractures of the distal forearm are the Colles' type.<sup>(29,30)</sup> For this first investigation to characterize genetic determination of OF, we choose to study Colles' fracture (CF), for the following reasons:

- (1) CF is one of the most prevalent OF.<sup>(27,31–34)</sup> CF generally is symptomatic and nearly always requires medical treatment. Therefore, confirmation of CF is relatively easy. CF accounts for a significant proportion of outpatient health resource utilization for OF treatment.<sup>(35)</sup> However, CF per se normally does not lead directly to markedly increased mortality and permanent morbidity, rendering it relatively easy to recruit study subjects with CF.
- (2) CF is predictive of underlying osteoporosis and subsequent OF.<sup>(36–38)</sup> A CF is indicative of an overall 50% increase in the risk of a subsequent hip fracture.<sup>(36)</sup> Women with CF have lower BMD at several skeletal sites, including spine, hip, and radius and have higher bone turnover rate.<sup>(37)</sup>
- (3) CFs in adults occur at relatively young ages, starting at approximately age 40 years. Many of the study subjects have live parents and siblings available. Information from these relatives is essential for many genetic studies.

To initiate extensive searches for genes underlying OF risk through the study of OF per se, direct evidence for the importance of the genetic determination of OF per se must first be provided. Especially, the genetic parameters that determine the likelihood of success of hunting for OF genes must be estimated. In this study, we will

- (1) Determine the prevalence ( $K$ ) of CF in a metropolitan white female population in the midwestern United States.
- (2) Determine the magnitude of genetic determination of CF as reflected by recurrence risks ( $K_R$ ) and relative risks ( $\lambda$ ) of CF.
- (3) Compare the bone mass in women with and without CF.

The terms  $K_R$ , and  $\lambda$  will be defined in detail in the Materials and Methods section. Their relationship to genetic variances of CF also will be given. Their role in the determination of the success of gene hunting for CF will be discussed. The prevalence of CF differs in males and females;<sup>(28)</sup> there also may exist differences of  $K_R$  and  $\lambda$  for different sex combinations, that is,  $K_R$  of a woman with an affected sister (a sister who has had CF) may be different from the  $K_R$  of a woman with an affected brother, partly because the prevalence of CF is much higher in females.<sup>(27,31–34)</sup> All the parameters (i.e.,  $K$ ,  $K_R$ , and  $\lambda$ ) will be estimated by a maximum likelihood approach that we develop here.

## MATERIALS AND METHODS

### Subjects and measurement

The data for this study were obtained during the preparatory part of an ongoing research involving a whole genome scan to detect genomic regions underlying the risk of CF, which was approved by the Creighton University Institutional Review Board. All study subjects signed informed-consent documents before entering.

The nuclear families of sisters and their mothers were ascertained. The probands came from a database containing all study subjects who have ever been participants of various bone studies or patients at the Osteoporosis Research Center of Creighton University. We mailed questionnaires to 3696 women from this database who were at least 40 years of age as of January 10, 1999 and inquired as to their CF status, the number of living sisters and their CF status, and finally the CF fracture status of their mothers. The mean ( $\pm$ SE) of the ages of the proband women was 65.55 (0.21). Throughout, unless otherwise specified, the number within parenthesis after an estimate is the associated SE. We received 2471 eligible responses as of April 1, 1999. The basic data are the information on the CF of the 2471 probands, 3803 sisters, and 2471 mothers of these probands. The total sample size is 8745. The CF status of these subjects is summarized in Table 1. Data from others<sup>(39–41)</sup>

TABLE 2. ESTIMATES OF PREVALENCE, RECURRENCE, AND RELATIVE RISKS

Prevalence			Recurrence risk		Relative risk	
$K_1$	$K_2$	$K_3$	$K_s$	$K_o$	$\lambda_s$	$\lambda_o$
0.118 (0.007)	0.044 (0.003)	0.147 (0.007)	0.084 (0.012)	0.155 (0.017)	1.885 (0.276)	1.312 (0.145)

The  $K_1$ ,  $K_2$ , and  $K_3$  are, respectively, the prevalence of CF in the probands, sisters of the probands, and mothers of the probands. The  $K_s$  and  $K_o$  are the recurrence risks of CF for sister-sister and daughter-mother pairs. The  $\lambda_s$  and  $\lambda_o$  are the relative risks of CF for sister-sister and daughter-mother pairs. The detailed definition of these parameters can be found in the Definition and statistical analysis subsection in the text.

and our own (Stegman MR, Deng HW, 1999, unpublished data) show that self-reported fractures are quite reliable, especially those generally requiring medical treatment, such as CF.

Many of the 3696 women to whom we sent mail had bone density measurements by dual-energy X-ray absorptiometry (DXA) at one or several of the following sites: spine, femoral neck, distal radius, and total body, together with records for age and weight at the time of bone density measurement. The spine was the combined BMD of L1-L4. Measurement by DXA at our center already has been described extensively previously (e.g., see Refs. 11, 21, and 25).

*Definitions and statistical analyses*

Because, to our knowledge, this is the first study to examine the genetic determination of OF per se, a brief introduction to the various measures of risk is in order. These measures of risk are defined in a more general way and in more detail by Lynch and Walsh.<sup>(42)</sup> We assigned a numerical value of 0 to a nonaffected individual (who has never had CF) and 1 to an affected individual (who has had CF; Table 2). Let  $K$  be the population prevalence of CF. If CF has a genetic basis, relatives of an affected individual should have a probability greater than  $K$  of being affected, simply because of the genetic determination of CF and genetic relatedness of relatives. Letting  $z_1$  and  $z_2$  denote the status of CF of two relatives, then the recurrence risk ( $K_R$ ) is the probability that a relative is affected given that the other is affected. The term  $R$  indicates the relationship between the two relatives.

$$K_R = \Pr(z_2 = 1 | z_1 = 1),$$

where “|” indicates a conditional probability. An alternative measure is the relative risk  $\lambda_R$ , the increase in risk that one relative is affected compared with the population prevalence  $K$ , when the other relative is affected,

$$\lambda_R = \frac{K_R}{K}.$$

Note, the definition of the relative risk here is different from that in the general field of epidemiology.<sup>(43)</sup> In addition,  $\lambda_R$  is a parameter scaled for the population prevalence  $K$ .

Because the incidence of CF is age dependent,<sup>(32)</sup> the prevalence ( $K$ ) of CF also should highly depend on the age groups of the subjects under study, as will be supported by our data here on the differential  $K$ 's in the groups of the mothers and the daughters. In addition, the probands are from the database created for the subjects who have been the patients or participants in studies conducted at our center. These probands are more likely to have osteoporosis or osteopenia and are more prone to OF than the general population. To account for potential difference in risks, we denote the different prevalence of CF in the probands, the sisters of the probands, and the mothers, respectively, as  $K_1$ ,  $K_2$ , and  $K_3$ . That is,

- $K_1$  = (the number of affected probands)/(the total number of probands);
- $K_2$  = (the number of affected sisters of the probands)/(the total number of sisters of the probands);
- $K_3$  = (the number of affected mothers of the probands)/(the total number of mothers of the probands).

The standard errors of  $K_1$ ,  $K_2$ , and  $K_3$  can be computed by the method of maximum likelihood, the principles of which will be outlined for a more complex situation for the estimation of  $\lambda_s$  and  $\lambda_o$  (Appendix 1). Although age data for the sisters and mothers of the probands are not available, on average as groups, there is no reason for the probands to differ in age from their sisters and mothers will have older ages than the daughters. However,  $K$ 's are likely to be different in probands and their sisters as reasoned earlier and will be verified later. The distinction of  $K$ 's in the probands and their sisters accounts for differential risks of CF in these two groups and thus accounts for the ascertainment through probands in the estimation developed in Appendix 1. The distinction of  $K$ 's in mothers and their daughters accounts for the differential risks in mothers and daughters simply because of the age difference and thus coarsely accounts for age dependence of the risks of CF in this study (also see Discussion).

For this study, let us define two recurrence risks as  $K_s = \Pr(\text{sister} = 1 | \text{proband} = 1)$ ,  $K_o = \Pr(\text{daughter} = 1 | \text{mother} = 1)$ , and define the two relative risks as  $\lambda_s = K_s/K_2$  and  $\lambda_o = K_o/K_2$ . In words,  $K_s$  is the probability that a sister of a proband will be affected with CF given that the proband is affected. The term  $K_o$  is the probability that a daughter will be affected conditional on her mother being affected. The term  $\lambda_s$  is the increase in risk of CF for a sister

TABLE 3. ESTIMATES OF ADDITIVE ( $\sigma_A^2$ ), DOMINANT ( $\sigma_D^2$ ) GENETIC VARIANCE, AND THE NARROW-SENSE HERITABILITY ( $h^2$ ) OF CF

$\sigma_A^2$	$\sigma_D^2$	$h^2$
0.0108 (0.0025)	-0.0029 (0.0058)	0.254 (0.118)

who has an affected sister compared with the prevalence  $K_2$  in the sister population. The term  $\lambda_0$  is the increase in risk of a daughter who has an affected mother relative to the population prevalence in the daughters. The recurrence risks and the relative risks and their SEs can be estimated by the maximum likelihood estimation developed in Appendix 1, which should be of general use for characterizing genetic determination of complex diseases.

Although recurrence ( $K_R$ ) and relative ( $\lambda_R$ ) risks are direct measures of the degree of genetic determination of complex diseases,<sup>(42)</sup> genetic variances and heritability ( $h^2$ ) are more familiar indices of genetic determination for continuous quantitative traits such as BMD. In addition, complex diseases may be modeled by continuously distributed quantitative traits (liabilities<sup>(42)</sup>) as threshold traits. Therefore, to help to see the relationship between prevalences ( $K$ 's) in different groups  $K_R$ ,  $\lambda_R$ , and the genetic variances and  $h^2$  we derived the relationship among them and developed a maximum likelihood estimation of additive ( $\sigma_A^2$ ) and dominant ( $\sigma_D^2$ ) genetic variances and  $h^2$  (Appendix 2) and estimated  $\sigma_A^2$ ,  $\sigma_D^2$ , and  $h^2$  (Table 3).

To compare bone mass in women with and without CF, we conducted multiple regression with bone mass as a dependent variable and age and weight as independent variables. The results are summarized in Table 4. We then used these multiple regression results to adjust bone mass for age and weight, which ensures that the differences of bone mass between women with and without CF will not be confounded by important covariates of age and weight.<sup>(21)</sup> The variances of adjusted bone mass data in women with and without CF were compared by F tests for homogeneity. Then the differences of the means of adjusted bone mass data were tested by appropriate *t*-tests. The results are summarized in Table 5. The differences of the standard Z scores at spine and femoral neck between groups of women with and without CF also were tested. The Z score denotes BMD in units of SDs above or below the mean of a healthy ethnic-, age-, and gender-matched referent population.

## RESULTS

The prevalences of CF are, respectively, 11.8% ( $\pm$ SE 0.7%) in the probands ( $K_1$ ), 4.4% (0.3%) in sisters of the probands ( $K_2$ ), and 14.6% (0.7%) in mothers of the probands ( $K_3$ ). The higher prevalence of CF in the mothers reflects the age dependence of the incidence of CF. With increasing age, the incidence of CF increases dramatically until a plateau is reached at  $\sim$ 60 years of age.<sup>(27)</sup> The higher prevalence of CF in the probands ( $K_1$ ) than in their sisters

( $K_2$ ) probably reflects the fact that the probands have been the participants of various bone studies to prevent osteoporosis or have been patients at our center. Therefore,  $K_1$  may be elevated relative to the same age group in the study population and  $K_2$  may reflect more closely the prevalence ( $K$ ) of the same age group in the population. Therefore, in the absence of the data from a random sample from the study population,  $K_2$  is employed to approximate  $K$  for women of  $\sim$ 65.6 years of age (0.21). However, it should be noted that  $K_2$  is still expected to be higher than  $K$  for the same age group in the study population, simply because of the relatedness of sisters to a selected group (probands) with higher risks of CF. Thus,  $K_2$  can be viewed as an upper boundary of the estimate of  $K$ . Importantly, it should be pointed out that analytically by the definitions of  $\lambda_R$ ,  $\lambda_s$ , and  $\lambda_0$  (in the subsection of Definition and statistical analyses in the Materials and Methods section and in Appendix 1), using  $K_1$  or  $K_2$  to substitute for  $K$  in the estimation will result in downward bias of the estimation of the true  $\lambda_s$  and  $\lambda_0$  values. Therefore, the estimated genetic parameters given below should be viewed as conservative estimates of the lower limits of the true values.

The recurrence risk (the probability of having CF) for a woman is 0.084 (0.012) given that she has a sister who has had CF ( $K_s$ ) and 0.155 (0.017) if her mother has had CF ( $K_0$ ). The relative risk of  $\lambda_s$  is 1.885 (0.276) and  $\lambda_0$  is 1.312 (0.145), both significantly greater than 1.0, indicating significant genetic determination in the occurrence of CF. Roughly speaking, a  $\lambda_s$  value of 1.885 indicates that the risk of CF for a woman with an affected sister is more than twice that of a random woman of similar age in the population. A  $\lambda_0$  value of 1.312 indicates that the risk of CF for a woman with an affected mother is about one and one-half times that of a random woman of similar age in the population.

When converted to the familiar index of genetic determination for continuous quantitative traits, the additive genetic variance ( $\sigma_A^2$ ) of CF is 0.0108 (0.0025) and the dominant genetic variance ( $\sigma_D^2$ ) is -0.0029 (0.0058). Thus, the  $\sigma_A^2$  is significant and  $\sigma_D^2$  is not statistically different from zero. Therefore, the genetic variance of CF is largely the heritable component  $\sigma_A^2$ . The narrow-sense heritability ( $h^2$ ) of CF is 0.254 (0.118), which indicates that  $\sim$ 25% of the variation of the occurrence of CF is determined genetically.

Age and weight had highly significant effects on BMD (Table 4), as is well recognized. Importantly, BMD of spine, femoral neck and wrist, and the total body bone mass were all significantly higher in women without CF than women with CF. The same conclusion held for the Z scores at spine and femoral neck. All the tests remained significant even after the multiple comparison was accounted for.

## DISCUSSION

To our knowledge, this study is the first that provides direct evidence for the magnitude of the genetic determination of CF—a common type of OF. Our results unambiguously indicate that there is a strong and moderately high degree of genetic determination of CF in white women. In addition, women without CF had significantly higher bone

TABLE 4. RESULTS OF MULTIPLE REGRESSION ANALYSES

	<i>Intercept</i>	<i>Age</i>	<i>Weight (kg)</i>	<i>Adj. R<sup>2</sup></i>
Spine BMD (g/cm <sup>2</sup> ) [2417]	0.8593	-0.0038	0.0042	0.22
Femoral neck BMD (g/cm <sup>2</sup> ) [1302]	0.7240	-0.0044	0.0032	0.34
Distal radius BMD (g/cm <sup>2</sup> ) [552]	0.7880	-0.0057	0.0026	0.42
Total body BMC (g) [392]	1783.1	-18.4	19.0	0.56

The numbers reported in this table are the partial regression coefficients, which are all significant at  $\alpha = 0.001$  level. The numbers within brackets are the sample sizes in multiple regression analyses.

TABLE 5. BONE MASS IN WOMEN WITH AND WITHOUT CF

	<i>Spine BMD</i> (g/cm <sup>2</sup> )	<i>Total body</i> <i>BMC (g)</i>	<i>Distal radius</i> <i>BMD (g/cm<sup>2</sup>)</i>	<i>Femoral neck</i> <i>DMD (g/cm<sup>2</sup>)</i>	<i>Z<sub>BMD</sub></i> <i>spine</i>	<i>Z<sub>BMD</sub></i> <i>femur neck</i>
Women with CF	0.82 (0.14) [233]	1600.6 (339.7) [50]	0.52 (0.08) [67]	0.59 (0.10) [107]	-0.12 (1.26) [233]	-0.84 (0.96) [107]
Women without CF	0.91 (0.17) [1528]	1938.0 (463.0) [272]	0.61 (0.12) [379]	0.67 (0.12) [904]	0.41 (1.49) [1528]	-0.41 (1.11) [904]
<i>p</i>	1.43E-11	0.0008	2.47E-05	2.26E-05	8.45E-09	4.28E-05

The numbers given are means, associated SDs (numbers within parentheses), and the sample sizes (numbers within brackets) for computing the mean and SDs. The *p* is the *p* value associated with respective *t*-tests for the differences between women with and without CF.

mass than those with CF. The approximation of *K* by *K*<sub>2</sub> (~4.4%) for CF in women ~65 years of age in our study population, although upwardly biased, is within the range of those estimates (~2–18%) obtained in different populations.<sup>(28–34,44–46)</sup> Population variation in the prevalence of OF has been well recognized before (e.g., see Refs. 28–34).

Our direct evidence of genetic determination of OF is consistent with several lines of earlier indirect evidence. First, there is racial difference in the incidence of OF.<sup>(27,47,48)</sup> This racial difference is shown to be at least partially related to the vitamin receptor D genotypes.<sup>(49)</sup> Second, within populations, COL1A1 gene polymorphisms are shown to be markers of vertebral fracture risk,<sup>(50)</sup> with the *Ss* and *ss* genotypes incurring a relative risk of 2.97. Third, family history is a strong predictor of risk of OF.<sup>(51–53)</sup> Particularly, the genetic determination of CF is consistent with the recent results suggesting several genomic regions underlying forearm BMD variation,<sup>(54)</sup> an important risk factor for CF.<sup>(37)</sup> The estimates for *K*<sub>0</sub>, *K*<sub>s</sub>,  $\lambda_0$ , and  $\lambda_s$ , have direct practical application for genetic counseling on the risks of CF for women who have sisters or mothers with CF. For example, the values of  $\lambda_0$  (1.312) and  $\lambda_s$  (1.885) clearly indicate that a woman with an affected sister or mother is predisposed genetically to an elevated risk of CF and should take preventive intervention for CF.

Prevention of OF is one central objective of bone studies. Genetic studies of bone largely have been confined to BMD. This is because BMD is an important risk factor for OF,<sup>(13–15)</sup> and BMD is relatively easy to measure.<sup>(55)</sup> However, genetics studies of OF are essential for the following reasons:

- (1) BMD is not the only important risk factor for OF. Many other identified and/or unidentified intrinsic factors also are important.<sup>(51–53,56–58)</sup> Many of these are under strong genetic control.<sup>(16–20)</sup> Importantly, genes underlying different risk factors are not all the same as reflected by the low genetic correlation between them.<sup>(11,58)</sup> In addition, many important risk factors may not yet have been identified, because no combination of the known risk factors can predict lifetime OF risk with high confidence.<sup>(51–53)</sup>
- (2) Measurements of BMD by current techniques may not be precise. For example, BMD often is measured by DXA, a projectional technique based on the two-dimensional projection of a three-dimensional structure. The values are expressed as bone content per unit area (g/cm<sup>2</sup>) of the projected image of the region of interest (ROI), which is only an approximation of the volumetric density. Correction factors for this are subject to error,<sup>(59–63)</sup> because there is no closed formula that defines the size of the vertebrae or the femur. Import-

tantly, DXA values are influenced by variation in the composition of soft tissues in the beam path of the ROI. Inhomogeneous fat distribution in soft tissues consisting of only 2 cm variation in the fat layer around the bone will influence DXA measurements by as much as 10%.<sup>(64)</sup>

- (3) Because of pleiotropic effects (i.e., the same gene controls multiple risk factors) that are common for complex traits,<sup>(42)</sup> alleles conferring high BMD may adversely affect other important aspects of bone and thus confer lower resistance to OF. It has been shown that a genetically homogeneous inbred mouse strain has higher bone mass but smaller bone size and is less sensitive in adapting to mechanical loading to increase stiffness of bone strength when compared with another inbred mouse strain.<sup>(65)</sup> Similarly, low BMD but more highly organized collagen fibrils actually may enhance bone mechanical strength and thus result in a lower risk for OF.<sup>(66)</sup>

Therefore, in addition to our effort to search for genes underlying individual risk factors such as bone mass, extensive efforts should be initiated to search for genes underlying OF through studying OF per se and to investigate the relevance and the importance to OF of the genes revealed for individual risk factors. Searching for genes underlying OF per se will assure that the genes discovered are important for the susceptibility to OF.

The moderately high genetic determination of CF indicates that searching for genes underlying CF is likely to be fruitful and, certainly, such effort should be warranted. The parameters involved in determining the likelihood of success of gene search are relative risks ( $\lambda$ ) for dichotomous complex diseases and  $h^2$  for continuous quantitative traits.<sup>(42,67-70)</sup> Generally, for quantitative trait (such as BMD), discovering a genetic locus responsible for more than 15% of phenotypic variation (i.e., the  $h^2$  due to this locus is greater than 0.15) is well within our current technical and analytical capabilities.<sup>(68-70)</sup> For complex diseases (such as OF), a locus that confers a relative risk of  $\lambda_s > 1.6$  also is well accomplishable.<sup>(67,71)</sup> To provide an intuitive comparison, we converted the standard measures (relative and recurrence risks) of genetic determination of complex dichotomous diseases to the more familiar index ( $h^2$ ) for continuous quantitative traits. The relative risk is 1.311 (0.145;  $\lambda_0$ ) for a woman with an affected mother and 1.885 (0.276;  $\lambda_s$ ) for a woman with an affected sister, which correspond to an  $h^2$  of 0.254 (0.118). Therefore, in light of both types of these measures, the prospect of searching for genes underlying the risk for CF is optimistic. This is especially true given that these estimates are the lower limits of the corresponding true trues (concordance) as indicated in the Results. Of course, the likelihood of success also depends on the genetic determination attributable to individual major genetic loci. However, genetic determination caused by individual major genetic loci will not be known before extensive and systematic molecular genetic studies are performed.

Except for spine fractures, almost all OFs result from low trauma, that is, a fall. Although we cannot specify exactly

how many, it is most likely that the majority of our CF cases are caused by low trauma as suggested by the significant difference of bone mass found between women with CF and those without CF in our sample. Inclusion of CF cases that are caused by accidental high trauma generally will reduce the chance to detect the difference of bone mass between women with and without CF and decrease the magnitude of genetic determination estimated, simply because of the randomness of accidents. Therefore, inadvertent inclusion of CF cases caused by accidental high trauma will render our estimation of genetic determination of CF even more conservatively lower than true values. CF probably has less of a relationship to BMD than other typical OFs at spine and hip and genes for various types of OF may not all be the same. However, consistent with the few earlier studies,<sup>(36,37)</sup> our data clearly show that CF is a strong indicator of the underlying low bone mass at all the skeletal sites examined. Thus, systematic molecular genetic studies such as a whole genome scan for genes underlying CF will have a scope broad enough to identify genes for non-BMD as well as BMD factors important in determining OF risk. Searching for genes underlying the risk of CF also should be important for prevention of osteoporosis and other types of OF. This is because CF is predictive of subsequent OF of other types and the underlying osteoporosis.<sup>(36-38)</sup> It should be noted that the genetic parameters obtained in this study have not been adjusted for many known nongenetic factors. The influence of nongenetic factors on the incidence of CF can be adjusted by employing techniques such as multiple logistic regression. Although the dependence of incidence of CF on age is coarsely accounted for by adjusting for various  $K$ 's in daughters and mothers, more accurate adjustment is possible by logistic regression if specific ages of most study subjects were known. Adjusting significant nongenetic factors can effectively control for the nongenetic causes in the incidence of CF and thus generally increase the apparent importance of major genes and the likelihood to detect them in genetic studies.<sup>(21,72)</sup>

Although commonly employed as the parameters to model dichotomous complex traits and to compute statistical power for search of genes underlying complex diseases, the estimation of  $K_0$ ,  $K_s$ ,  $\lambda_0$ , and  $\lambda_s$  has been rare for many disease traits. Particularly, although the definitions of these parameters are simple, their estimation is not trivial in practice with complex family structure. The maximum likelihood method developed here can estimate not only the means but also the variances of the  $K_0$ ,  $K_s$ ,  $\lambda_0$ ,  $\lambda_s$ ,  $\sigma_A^2$ ,  $\sigma_D^2$ , and  $h^2$  of complex diseases. The method is general and can be applied directly or extended to characterize genetic determination of any complex disease based on nuclear families.

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#### APPENDIX 1. ESTIMATION OF THE RECURRENCE AND RELATIVE RISKS BY MAXIMUM LIKELIHOOD

Recall that from the text, the numerical value of 1 indicates that an individual is affected with CF and 0 indicates that she is unaffected. The terms  $K_1$ ,  $K_2$ , and  $K_3$  are,



respectively, the prevalence of CF in the probands, the sisters of the probands, and the mothers of the probands. Mathematically,

$$K_1 = \text{Pr}(\text{proband} = 1),$$

$$K_2 = \text{Pr}(\text{sister} = 1),$$

$$K_3 = \text{Pr}(\text{mother} = 1),$$

where Pr indicate a probability. Also defined in the text are

$$K_s = \text{Pr}(\text{sister} = 1 | \text{proband} = 1),$$

$$K_0 = \text{Pr}(\text{proband} = 1 | \text{mother} = 1),$$

$$\lambda_0 = K_0 / K_1,$$

and

$$\lambda_s = K_s / K_2.$$

For  $\lambda_0$ , we have

$$\begin{aligned} \lambda_0 &= K_0 / K_1 \\ &= \text{Pr}(\text{proband} = 1 | \text{mother} = 1) / \text{Pr}(\text{proband} = 1) \\ &= \frac{\text{Pr}(\text{proband} = 1, \text{mother} = 1)}{\text{Pr}(\text{proband} = 1) * \text{Pr}(\text{mother} = 1)} \\ &= \frac{\text{Pr}(\text{mother} = 1 | \text{proband} = 1) * \text{Pr}(\text{proband} = 1)}{\text{Pr}(\text{proband} = 1) * \text{Pr}(\text{mother} = 1)} \\ &= \text{Pr}(\text{mother} = 1 | \text{proband} = 1) / K_3 \\ &= K'_0 / K_3, \end{aligned}$$

where  $K'_0 = \text{Pr}(\text{mother} = 1 | \text{proband} = 1)$ . Therefore, for computational convenience, we will compute  $\lambda_0$  via  $K'_0 / K_3$ .

For any subject in the sample, she is either a proband or a sister of a proband or a mother of a proband; furthermore, she is either affected or unaffected with CF. Therefore, conditional on the CF status of a proband, we can express various CF status of her sister or mother using the parameters defined earlier as

$$\text{Pr}(\text{sister} = 1 | \text{proband} = 1) = K_s = K_2 \lambda_s,$$

$$\text{Pr}(\text{sister} = 0 | \text{proband} = 1) = 1 - K_s = 1 - K_2 \lambda_s,$$

$$\begin{aligned} \text{Pr}(\text{sister} = 1 | \text{proband} = 0) &= \frac{\text{Pr}(\text{sister} = 1, \text{proband} = 0)}{\text{Pr}(\text{proband} = 0)} \\ &= \frac{\text{Pr}(\text{sister} = 1) - \text{Pr}(\text{sister} = 1, \text{proband} = 1)}{1 - \text{Pr}(\text{proband} = 1)} \\ &= \frac{\text{Pr}(\text{sister} = 1) - \text{Pr}(\text{proband} = 1) * \text{Pr}(\text{sister} = 1 | \text{proband} = 1)}{1 - K_1} \end{aligned}$$

$$= \frac{K_2 - K_1 * K_s}{1 - K_1},$$

$$\text{Pr}(\text{sister} = 0 | \text{proband} = 0)$$

$$= 1 - \text{Pr}(\text{sister} = 1 | \text{proband} = 0) = 1$$

$$- \frac{K_2 - K_1 * K_2 \lambda_s}{1 - K_1}.$$

Similarly,

$$\text{Pr}(\text{mother} = 1 | \text{proband} = 1) = K'_0 = K_3 \lambda_0,$$

$$\text{Pr}(\text{mother} = 0 | \text{proband} = 1) = 1 - K_3 \lambda_0,$$

$$\text{Pr}(\text{mother} = 1 | \text{proband} = 0) = \frac{K_3 - K_1 * K_3 \lambda_0}{1 - K_1},$$

$$\text{Pr}(\text{mother} = 0 | \text{proband} = 0) = 1 - \frac{K_3 - K_1 * K_3 \lambda_0}{1 - K_1}.$$

Let  $I$  be an index variable, so that  $I = 0$  indicates that a mother is unaffected and  $I = 1$  indicates that the mother is affected with CF. Then, the probability that in the  $i$ th family, the proband is affected, her  $n_i$  sisters are affected and  $m_i$  sisters are unaffected is

$$C_{n_i + m_i}^{m_i} (\lambda_s K_2)^{n_i} (1 - \lambda_s K_2)^{m_i} (\lambda_0 K_3)^{I_i} (1 - \lambda_0 K_3)^{1 - I_i},$$

where  $C_{n_i + m_i}^{m_i}$  is the number of various combinations of choosing  $m_i$  individuals out of the total of  $(m_i + n_i)$  individuals. The probability that in the  $i$ th family, the proband is not affected, her  $n_i$  sisters are affected, and  $m_i$  sisters are unaffected is

$$\begin{aligned} C_{n_i + m_i}^{m_i} &\left( \frac{K_2 - K_1 K_2 \lambda_s}{1 - K_1} \right)^{n_i} \left( 1 - \frac{K_2 - K_1 K_2 \lambda_s}{1 - K_1} \right)^{m_i} \\ &\times \left( \frac{K_3 - K_1 K_3 \lambda_0}{1 - K_1} \right)^{I_i} \left( 1 - \frac{K_3 - K_1 K_3 \lambda_0}{1 - K_1} \right)^{1 - I_i}. \end{aligned}$$

Therefore, the likelihood ( $L$ ) of the data is the product of these probabilities across the families of the 2396 probands. Given our data as indicated in Table 1, we have

$$\begin{aligned} L &= C (\lambda_s K_2)^{32} (1 - \lambda_s K_2)^{341} (\lambda_0 K_3)^{56} (1 - \lambda_0 K_3)^{237} \\ &* \left( \frac{K_2 - K_1 K_2 \lambda_s}{1 - K_1} \right)^{137} \left( 1 - \frac{K_2 - K_1 K_2 \lambda_s}{1 - K_1} \right)^{3293} \\ &\times \left( \frac{K_3 - K_1 K_3 \lambda_0}{1 - K_1} \right)^{304} \left( 1 - \frac{K_3 - K_1 K_3 \lambda_0}{1 - K_1} \right)^{1874}, \quad (1) \end{aligned}$$

where  $C$  is a constant for the product of the coefficients, in which the magnitude is not important in the maximum likelihood estimation. Given that  $K_1$ ,  $K_2$ , and  $K_3$  can be easily estimated from Table 1, the maximum likelihood estimates of  $\lambda_s$  and  $\lambda_0$  and their variance can be obtained by standard methods via the first and second derivatives of the

likelihood function  $L$  with respect to  $\lambda_s$  and  $\lambda_0$ .<sup>(73)</sup> Briefly, the maximum likelihood estimates of  $\lambda_s$  and  $\lambda_0$  are the values that simultaneously satisfy the equations

$$\frac{\partial L}{\partial \lambda_s} = 0$$

and

$$\frac{\partial L}{\partial \lambda_0} = 0,$$

where  $\partial L/\partial \lambda_s$  indicates the first partial derivative of  $L$  with respect to  $\lambda_s$ , and  $\partial L/\partial \lambda_0$  is similarly defined. The variance of  $\lambda_s$  is the value of  $-1/\{\partial^2 \text{Ln}(L)/\partial \lambda_s^2\}$  evaluated at the maximum likelihood estimates of  $\lambda_s$  and  $\lambda_0$ . A similar approach can be adopted to write the likelihood of the whole data as functions of  $K_s$  and  $K_0$  to obtain the maximum likelihood estimates and the associated SDs of  $K_s$  and  $K_0$ .

## APPENDIX 2. ESTIMATION OF THE ADDITIVE ( $\sigma_A^2$ ), DOMINANT ( $\sigma_D^2$ ) GENETIC VARIANCE, AND NARROW-SENSE HERITABILITY ( $h^2$ ) OF CF

Genetic variances of CF can be estimated by using the observed recurrence or relative risks and population prevalence of diseases for different sets of relatives.<sup>(42,74,75)</sup> However, the basic method developed by these authors does not allow for the different prevalence of CF in different groups. Therefore, we generalize their method to accommodate the situation of different prevalence of CF in different groups, which is a practical situation in studies of diseases for which the occurrence of the diseases differ in different age or sex groups. Thus, our extension here should be of some general utility.

Let  $P$  denote the status of CF for the proband daughter and  $M$  for the mother of the proband. Again, we use 1 to denote the affected status and 0 for the unaffected status. Because  $P$  and  $M$  are 0-1 indicator variables,  $P * M = 0$  unless  $P = M = 1$ ; hence, the covariance of the occurrence of CF in the proband daughter and her mother is

$$\begin{aligned} \text{Cov}(P, M) &= E(PM) - E(P) * E(M) \\ &= \text{Pr}(P = 1, M = 1) - \text{Pr}(P = 1) * \text{Pr}(M = 1) \\ &= \text{Pr}(P = 1|M = 1) * \text{Pr}(M = 1) - K_1 K_3 \\ &= K_0 K_3 - K_1 K_3 \\ &= K_3 K_1 \lambda_0 - K_1 K_3 = K_1 K_3 (\lambda_0 - 1). \end{aligned}$$

Similarly, the covariance between proband and her sister ( $S$ ) is

$$\text{Cov}(P, S) = K_1 K_2 (\lambda_s - 1).$$

From the principles of quantitative genetics,<sup>(42)</sup> we know that

$$\text{Cov}(P, M) = \sigma_A^2/2$$

and

$$\text{Cov}(P, S) = \sigma_A^2/2 + \sigma_D^2/4.$$

Therefore, we have

$$\sigma_A^2 = 2K_1 K_3 (\lambda_0 - 1) \quad (1a)$$

and

$$\sigma_D^2 = 4K_1 K_2 (\lambda_s - 1) - 2\sigma_A^2. \quad (1b)$$

The narrow-sense heritability ( $h^2$ ) is defined<sup>(42)</sup> as

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2},$$

where  $\sigma_P^2$  is the phenotypic variance of CF, which can be obtained for different groups by the prevalence ( $K$ ) of that group:

$$\sigma_P^2 = K * (1 - K).$$

Therefore, in the sisters of the proband, the  $h^2$  for CF is

$$h^2 = \frac{2K_1 K_3 (\lambda_0 - 1)}{K_2 (1 - K_2)}. \quad (2)$$

In the likelihood function [Eq. (1) in Appendix 1], if we substitute  $\lambda_0$  with  $h^2$  using the above relationship, the maximum likelihood estimate and its variance for  $h^2$  can be obtained by standard means as outlined in the Appendix 1. To obtain the maximum likelihood estimates of  $\sigma_A^2$ ,  $\sigma_D^2$ , and their variances, the same procedure can be adopted to substitute  $\lambda_0$  and  $\lambda_s$  with  $\sigma_A^2$  and  $\sigma_D^2$  [using the relationship of Eqs. (1a) and (1b)] into the likelihood function [Eq. (1) of Appendix 1].