Differential Genetic Effects of *ESR1* Gene Polymorphisms on Osteoporosis Outcomes

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Context Both bone mineral density (BMD) and fracture risk have a strong genetic component. Estrogen receptor α (*ESR1*) is a candidate gene for osteoporosis, but previous studies of *ESR1* polymorphisms in this field were hampered by small sample size, lack of standardization, and inconclusive results.

Objective To generate large-scale evidence on whether 3 common *ESR1* polymorphisms (intron 1 polymorphisms *Xba*I [dbSNP: rs9340799] and *Pvu*II [dbSNP: rs2234693] and promoter TA repeats microsatellite) and haplotypes thereof are associated with BMD and fractures.

Design and Setting Meta-analysis of individual-level data involving standardized genotyping of 18 917 individuals in 8 European centers.

Main Outcome Measures BMD of femoral neck and lumbar spine; all fractures and vertebral fractures by genotype.

Results No between-center heterogeneity was observed for any outcome in any genetic contrast. None of the 3 polymorphisms or haplotypes had any statistically significant effect on BMD in adjusted or unadjusted analyses, and estimated differences between genetic contrasts were 0.01 g/cm^2 or less. Conversely, we found significant reductions in fracture risk. In women homozygous for the absence of an *Xba*I recognition site, the adjusted odds of all fractures were reduced by 19% (odds ratio, 0.81 [95% CI, 0.71-0.93]; *P*=.002) and vertebral fractures by 35% (odds ratio, 0.65 [95% CI, 0.49-0.87]; *P*=.003). Effects on fractures were independent of BMD and unaltered in adjusted analyses. No significant effects on fracture risk were seen for *Pvu*II and TA repeats.

Conclusions *ESR1* is a susceptibility gene for fractures, and *Xba*I determines fracture risk by mechanisms independent of BMD. Our study demonstrates the value of adequately powered studies with standardized genotyping and clinical outcomes in defining effects of common genetic variants on complex diseases.

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OSTEOPOROSIS IS A COMMON disease characterized by reduced bone mass and an increased risk of fracture, which affects up to 30% of women and 12% of men at some point during life. Bone mineral density (BMD) is an important clinical predictor of fracture risk, and most of the variance in BMD is genetically determined.[1,2] Many other predictors of fragility fracture are also under genetic control, however, including ultrasound properties of bone, biochemical markers of bone turnover, and skeletal geometry. A wide variety of candidate genes have been investigated in relation to osteoporosis outcomes, but one of the most widely studied is the

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estrogen receptor α (*ESR1*) gene.[3] In particular, polymorphisms defined by the restriction enzymes *Xba*I (dbSNP [database of single-nucleotide polymorphisms]: rs9340799) and *Pvu*II (dbSNP: rs2234693) in the first intron of *ESR1* have been evaluated to date in approximately 40 studies, with inconclusive results. These 2 polymorphisms are 46 base pairs apart and in strong linkage disequilibrium with a microsatellite TA-variable number of tandem repeats (VNTR) polymorphism[4] (dbSNP: rs3138774) situated 2.1 kb upstream in the *ESR1* promoter region. The role of this VNTR in osteoporosis outcomes is controversial, and interpretation is further limited by analytic inconsistencies across published reports.[4-9]

There is increasing recognition that, given the common lack of replication of results of small studies,[10-12] the delineation and establishment of common genetic risk factors for complex multigenetic disorders, such as osteoporosis, requires large-scale investigations to clarify subtle, but clinically important, genetic effects.[12,13] Standardization is also essential to avoid misinterpreting as genuine genetic variability whatever differences between study teams are caused by analytical inconsistencies. We report the results of a collaborative study using standardized genotyping methodology on 18 917 individuals, which tests the contribution of these 3 common *ESR1* polymorphisms and haplotypes thereof on BMD and fractures.

METHODS

The GENOMOS (Genetic Markers for Osteoporosis) project involves the study of several candidate gene polymorphisms in relation to osteoporosis-related outcomes in approximately 20000 individuals drawn from 8 European centers.[14] Participating teams contributed information on sex, age, height, weight, TA genotype (number of TA repeats in each allele), *Xba*I genotype, *Pvu*II genotype, BMD at lumbar spine (L2-4) and femoral neck (in g/cm²), fractures at any site, vertebral fractures, and menopausal status.

The 2 largest cohorts in the meta-analysis (Rotterdam and Aberdeen)

genotyped their entire population, whereas other cohorts generally excluded women with secondary causes of osteoporosis or those receiving drugs that could affect bone metabolism. Study design aspects for each cohort in the consortium are available from the author on request. All participating centers have received institutional review board or ethics committee approval according to their local regulations, and participant informed consent has been obtained according to the requirements of each center.

Genotyping

Genotyping for the 3 polymorphisms was performed in different centers by using polymerase chain reaction–restriction fragment-length polymorphism, single-base extension sequencing and 5′ nuclease Taqman assays for the *Xba*I and *Pvu*II polymorphisms and capillary electrophoresis for TA-VNTR.[5-7] For *Xba*I and *Pvu*II, *X* and *P* denote the absence of the respective restriction sites (*G* allele and *C* allele, respectively).

Each center checked its own genotyping by reanalyzing at least 5% of the samples with random selection. To ensure standardization between centers, 50 randomly selected samples from 1 center (Rotterdam) were sent in blinded fashion to all the other cohorts for independent analysis. Results were assembled and compared at the coordinating center. For *Xba*I and *Pvu*II, only 1 sample gave discrepant results for *Xba*I in 1 cohort. For the TA repeats, 2 cohorts systematically estimated 1 fewer repeat, and 1 estimated systematically 2 fewer repeats. Thus, readings were adjusted in these cohorts by adding 1 or 2 repeats, respectively. Aside from these systematic differences, 21 of the allele determinations across cohorts did not agree exactly with the predominant determination, but with the exception of 6 alleles (error rate <1%), the difference was less than 4 repeats. No data were obtained for TA repeats in one study (Cambridge), whereas in another study (Florence) TA repeats had been determined with a different method that showed extensive differences in the pilot samples.

Thus, these data were not considered in any analyses. Hardy-Weinberg equilibrium was checked on all data.

BMD Measurements

Bone mineral density was assessed by dual-energy x-ray absorptiometry with Hologic devices in the Barcelona, DOPS (Danish Osteoporosis Study), Aarhus, and Florence studies, Norland in the Aberdeen study, Lunar DPX-L in Rotterdam, and a variety of devices crosscalibrated with the European Spine Phantom in the Oxagen and Cambridge cohorts.[15] Syntheses of BMD data across studies always include also a study effect that would account both for genuine differences in populations and potential systematic differences between these devices. The results of the meta-analysis for BMD should be interpreted with emphasis on the BMD differences between the contrasted genotypes and haplotypes and not on the absolute BMD values.

Definitions and Outcomes

We analyzed genotypes for each of the 3 polymorphisms and long-range haplotypes (LRHs) by combining all 3 polymorphisms. The microsatellite genotypes were clustered in 2 groups of alleles according to the bimodal appearance of the composite distribution of the number of repeats.[4,5] The low-repeat number group (*L*) was defined to extend up to the trough of the distribution, and alleles with higher numbers of repeats were grouped in the high-repeat number group (*H*). The resulting genotypes are *HH*, *HL*, and *LL*. Long-range haplotypes (*x-p-L* [A], *X-P-L* [B], *x-P-L* [C], *x-p-H* [D], *X-P-H* [E], *x-P-H* [F], *X-p-H* [G], and *X-p-L* [H]) were imputed by using the PHASE program.[16]

The main outcomes included lumbar spine BMD; femoral neck BMD; any recorded fractures based on clinical history or radiographic evaluation, as defined in each study; and vertebral fractures based on clinical or radiographic evaluation, according to the criteria of McCloskey et al.[17] Prevalent fractures (at BMD determination) were considered in all cohorts. Data on incident fractures

during prospective follow-up were also collected according to clinical history for peripheral fractures and comparison of spine radiographs at follow-up (average, 7.4 years) vs baseline on 3469 participants in the Rotterdam cohort, clinical history in the Aberdeen cohort, and clinical history for peripheral fractures and spine radiographs in the small Cambridge sample. Four cohorts (Florence, Barcelona, Aarhus, and Cambridge) consistently excluded up-front fractures caused by high-energy trauma. In 3 of the 4 remaining cohorts (Rotterdam, Oxagen, and DOPS), we could also separate fractures without obvious trauma (typically vertebral fractures observed on radiographs) and low-energy trauma from those caused by high-energy trauma according to location (face, distal foot, distal hand) or medical history (injury, fall from height, impact sports). This separation was not possible in Aberdeen. Genotyping was performed blinded to the clinical data and vice versa.

For all analyses, data in each cohort were first split according to sex. In all studies participants were unrelated, with the exception of Oxagen pedi-

Table 1. Study Population Characteristics*

|	Aarhus	Aberdeen	Barcelona	Cambridge		DOPS	Florence	Oxagen		Rotterdam	
Sex	Women (n = 584)	Women (n = 3886)	Women (n = 558)	Women (n = 230)	Men (n = 95)	Women (n = 1703)	Women (n = 973)	Women (n = 1810)	Men (n = 1068)	Women (n = 4878)	Men (n = 3105)
Age, mean (SD), y	61.4 (13.1)	48.5 (2.4)	53.7 (8.4)	64.9 (7.5)	66.3 (8.2)	50.1 (2.8)	60.0 (10.0)	49.2 (15.3)	48.9 (16.0)	70.1 (9.3)	68.4 (8.2)
Height, mean (SD), cm	161.5 (6.5)	160.9 (8.7)	156.6 (6.3)	160.0 (6.4)	168.9 (5.7)	164.5 (7.2)	158.9 (6.6)	163.1 (7.1)	177.3 (7.4)	161.1 (6.7)	174.6 (6.8)
Weight, mean (SD), kg	64.1 (10.8)	66.2 (12.2)	64.3 (10.5)	67.4 (11.9)	77.9 (10.9)	67.8 (12.1)	63.5 (10.5)	65.2 (12.3)	80.4 (12.5)	69.3 (11.4)	78.2 (10.8)
BMI, mean (SD)†	24.6 (4.1)	25.5 (4.5)	26.2 (3.9)	26.3 (4.3)	27.3 (3.5)	25.0 (4.3)	25.2 (4.2)	24.5 (4.3)	25.6 (3.6)	26.7 (4.1)	25.6 (3.0)
Postmenopausal, No. (%)	484 (82.9)	1779 (46.1)	554 (100)	230 (100)	NA	1703 (100)	939 (96.6)	969 (53.4)	NA	4865 (99.7)	NA
Hormone therapy, No. (%)‡	0 (0.0)	2266 (58.6)	0 (0.0)	33 (14.3)	NA	0 (0.0)	107 (11.0)	457 (25.2)	NA	692 (15.0)	NA
Activity/ability, median (range)§	. . .	1.77 (0.76-3.26)	. . .	21 (7-24)	22 (7-24)	15 (0-80)	. . .	7.33 (3-14)	8.00 (3-14)	2.75 (0-3)	2.88 (0-3)
BMD											
Method	Hologic (cal)	Norland	Hologic	Various (ESP)	Various (ESP)	Hologic	Hologic	Various (ESP)	Various (ESP)	Lunar	Lunar
Lumbar spine No.	564	3882	554	47	21	1699	948	1753	1034	3381	2463
Mean (SD), g/cm²	0.827 (0.179)	1.052 (0.161)	0.877 (0.149)	1.008 (0.224)	1.058 (0.255)	1.026 (0.138)	0.867 (0.188)	0.970 (0.211)	1.071 (0.219)	1.036 (0.180)	1.165 (0.197)
Femoral neck No.	558	3881	423	213	75	1691	572	1776	1047	3239	2435
Mean (SD), g/cm²	0.671 (0.129)	0.881 (0.125)	0.695 (0.109)	0.747 (0.151)	0.872 (0.171)	0.796 (0.115)	0.662 (0.134)	0.761 (0.151)	0.847 (0.159)	0.811 (0.131)	0.876 (0.133)
Fractures, No. (%)											
Any	247 (42.3)	1216 (31.3)	54 (19.6)	61 (26.5)	27 (28.4)	342 (20.1)	207 (21.3)	748 (41.9)	508 (48.3)	1149 (23.6)	385 (12.4)
No/low trauma	247 (42.3)	. . .	54 (19.6)	61 (26.5)	27 (28.4)	121 (7.1)	207 (21.3)	466 (25.7)	236 (22.1)	890 (18.3)	219 (7.1)
Vertebral	247 (42.3)	21 (0.5)	31 (11.3)	52 (22.6)	26 (27.4)	29 (1.7)	122 (12.5)	105 (5.9)	53 (5.0)	239 (12.1)	142 (9.5)
XbaI genotype, No. (%)											
XX	62 (10.6)	347 (12.1)	74 (13.9)	32 (14.0)	10 (10.9)	166 (10.2)	126 (13.4)	221 (12.8)	126 (12.3)	475 (12.3)	305 (11.7)
Xx	269 (46.2)	1333 (46.3)	260 (48.8)	97 (42.5)	43 (46.7)	759 (46.5)	441 (46.8)	772 (44.7)	458 (44.7)	1725 (44.7)	1204 (46.0)
xx	251 (43.1)	1196 (41.6)	199 (37.3)	99 (43.4)	39 (42.4)	709 (43.4)	375 (39.8)	733 (42.5)	440 (43.0)	1663 (43.0)	1109 (42.4)
PvuII genotype, No. (%)											
PP	128 (22.0)	646 (22.4)	112 (21.0)	48 (21.1)	18 (18.9)	321 (19.7)	174 (18.5)	376 (21.8)	215 (21.2)	837 (21.7)	566 (21.6)
Pp	291 (50.1)	1385 (48.1)	261 (49.0)	118 (51.8)	42 (44.2)	827 (50.7)	483 (51.4)	834 (48.3)	501 (49.4)	1894 (49.0)	1322 (50.4)
pp	162 (27.9)	848 (29.5)	160 (30.0)	62 (27.2)	35 (36.8)	483 (29.6)	283 (30.1)	516 (29.9)	298 (29.4)	1132 (29.3)	733 (28.0)
TA repeats, median (IQR)	16 (15-23)	17 (15-23)	16 (15-23)	. . .	. . .	16 (15-23)	. . .	16 (15-22)	16 (15-23)	17 (15-23)	17 (15-23)
TA genotype, No. (%)											
HH	128 (22.5)	650 (22.0)	102 (18.9)	. . .	. . .	339 (20.1)	. . .	214 (16.5)	142 (18.9)	415 (22.1)	258 (21.1)
HL	274 (48.1)	1437 (48.6)	256 (47.5)	. . .	. . .	815 (48.3)	. . .	621 (47.8)	347 (46.3)	907 (48.3)	600 (49.0)
LL	168 (29.5)	870 (29.4)	181 (33.6)	. . .	. . .	532 (31.6)	. . .	463 (35.7)	261 (34.8)	556 (29.6)	366 (29.9)

Abbreviations: BMD, bone mineral density; cal, calibration of a few Norland values to Hologic equivalents; DOPS, Danish Osteoporosis Study; ESP, European Spine Phantom calibration; HH, both alleles with >18 repeats; HL, heterozygotes; LL, both alleles with ≤18 repeats; NA, not applicable; ellipses, no data available.
*Reported percentages are estimated based on individuals with available data for the respective characteristic. All Oxagen data are shown (not just those pertaining to the randomly selected sample including only 1 subject per pedigree). Data on 27 men from Florence are not shown here and were not included in the data synthesis, given the very small sample size.
†Measured as weight in kilograms divided by the square of height in meters.
‡At any time up to BMD measurement.
§Physical activity/ability data were measured in different scales capturing various abilities (in the Rotterdam and Cambridge cohorts) and/or activities (in the Aberdeen, Oxagen, DOPS, and Cambridge cohorts), and thus the scales and range of values are different, so they should not be compared between cohorts. All adjusted analyses using these indices were performed first within each cohort, and then the adjusted estimates were synthesized.

Figure 1. Distribution of Microsatellite Alleles
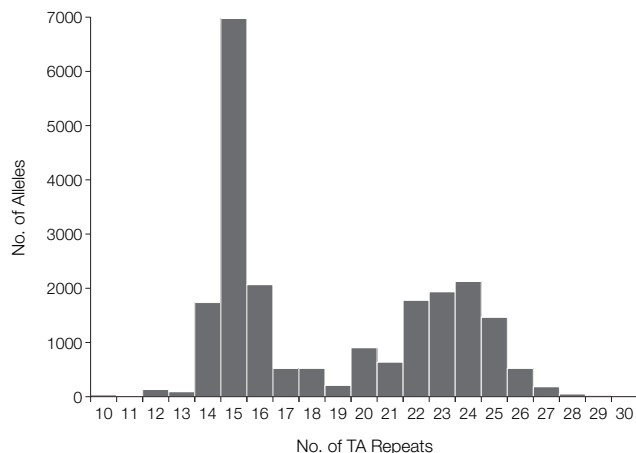
Microsatellite alleles according to the number of TA repeats in the cumulative meta-analysis population for the following sites: Aarhus, Aberdeen, Barcelona, DOPS (Danish Osteoporosis Study), Oxagen, and Rotterdam.

grees. For analytic consistency, in the main data synthesis we used only 1 randomly selected individual per Oxagen pedigree. Sensitivity analysis using all Oxagen participants yielded largely similar results (data not shown).

Analyses

For each genotype of interest, we estimated the unadjusted mean BMD and standard deviation in each study. We then synthesized BMD differences between genotype contrasts across studies by using fixed- and random-effects general variance models.[18] Between-study heterogeneity was assessed by the Q statistic (considered significant for $P<.10$). Random-effects models incorporated the between-study heterogeneity and allowed for a different effect in each population.[18] In the absence of between-study heterogeneity, fixed and random effects are similar.

We also performed analyses adjusting for the potential independent effect of each polymorphism, as well as age, weight, and height, plus menopausal status and any hormone therapy for women. Separate adjusted analyses were performed for the genotypes of each polymorphism and for the combinations of LRHs stemming from the 2 most common LRHs (A and E), ie, comparing individuals with 2, 1, or no copies of haplotype A and with 2, 1, or no copies of

haplotype E. We considered study as a random factor and allowed study × genotype (or haplotype) interactions to account for potentially variable genetic effects across studies. The overall significance of the genetic effects was evaluated with an F test for between-participant effects. Marginal means were also obtained. *P* values estimated for the comparison of estimated marginal means tended to be smaller and should be interpreted with more caution.

For fractures, we estimated the number of individuals in each genotype and haplotype group of interest, and pairwise genotype and haplotype comparisons were performed by estimate of an odds ratio (OR) in each study. Genotype analyses investigated recessive and dominant models for each polymorphism, and haplotype contrasts were based on the 2 most common LRHs (A and E). In each analysis, ORs were evaluated for between-study heterogeneity by using the Q statistic (considered significant for $P<.10$) and then synthesized with the Mantel-Haenszel (fixed-effects) and DerSimonian and Laird (random-effects) methods.[18] We also performed sensitivity analyses limited to incident fractures, limited to no-trauma/low-energy-trauma fractures, and limited to women who had not received hormone therapy. Adjusted logistic regression analyses were also performed by

considering age, height, weight, and menopausal status for women, as well as BMD. Sensitivity analyses were performed also for the adjusted estimates after further adjusting for indices of physical activity and ability. Data on different such indices were available for the 4 largest cohorts (Rotterdam, Aberdeen, Oxagen, and DOPS) and for Cambridge.

Analyses were conducted using SPSS version 11.0 (SPSS Inc, Chicago, Ill) and Meta-Analyst (Joseph Lau, Boston, Mass). All reported *P* values are 2-tailed and unadjusted for multiple comparisons.

RESULTS

Assembled Database

Data on 18 917 individuals were assembled, of whom 14 622 were women (3555 with current or past use of hormone therapy) (**TABLE 1**). Data on lumbar spine BMD, femoral neck BMD, any fractures, and vertebral fractures were available on 16 370, 15 926, 18 841, and 14 039 participants, respectively. Across the database, age quartile cutoffs were 47.4, 50.6, 58.0, and 69.0 years for women and 56.5, 62.1, 67.3, and 73.8 years for men. The database included 4952 individuals with any fracture and 1072 with vertebral fractures. There were 1779 individuals with incident fractures, the vast majority derived from the Rotterdam (n=1260) and Aberdeen cohorts (n=489). Only the Rotterdam cohort had a meaningful number of analyzable incident radiographically screened vertebral fractures (n=176). There were 2536 participants with no-trauma/low-trauma fractures across the 7 cohorts with relevant data (excluding Aberdeen). Standardized data on *Xba*I, *Pvu*II, and TA repeat genotypes were obtained in 16 147, 16 135, and 10 902 individuals, respectively (Table 1). All 3 polymorphisms were in strong linkage disequilibrium with each other. The distribution of TA repeats was consistently bimodal in all studies, and overall the trough of the distribution was clearly seen at 19 repeats (**FIGURE 1**). For all cohorts, the A haplotype (*x-p-L*) accounted consistently for about half of the alleles (range, 47.1% to 53.4%) and the E haplotype (*X-P-H*) for al-

most a third (range, 29.6% to 32.4%). (Frequencies of inferred LRHs per cohort are available from the corresponding author on request.)

Bone Mineral Density

In unadjusted analyses, none of the 3 polymorphisms was statistically significantly associated with BMD in the lumbar spine or in the femoral neck for any of the tested genotype contrasts (**FIGURE 2** and **FIGURE 3**), with the ex-

ception of a slightly higher femoral neck BMD with *XX* as compared with *xx* (statistically significant at *P*<.05 by fixed-effects only). There was no statistically significant between-study heterogeneity for any of the comparisons (heterogeneity *P*>.10 for all). The estimated differences in BMD were 0.01 g/cm^2 or less for all genetic contrasts (Figure 2 and Figure 3). The results were similar when limited to women only, with no significant between-

study heterogeneity and maximal estimated differences in the same range. The more sparse data on men were consistent with this picture, but estimates had more uncertainty (Figure 2 and Figure 3). Analyses adjusted for age, height, weight, hormone therapy, and menopausal status also showed that none of the 3 polymorphisms had a statistically significant association with BMD (not shown in detail). The typical trend for all these analyses in-

Figure 2. Differences in Lumbar Spine Bone Mineral Density (BMD) for the Contrasts of *Xba*I, *Pvu*II, and TA Repeat Genotypes
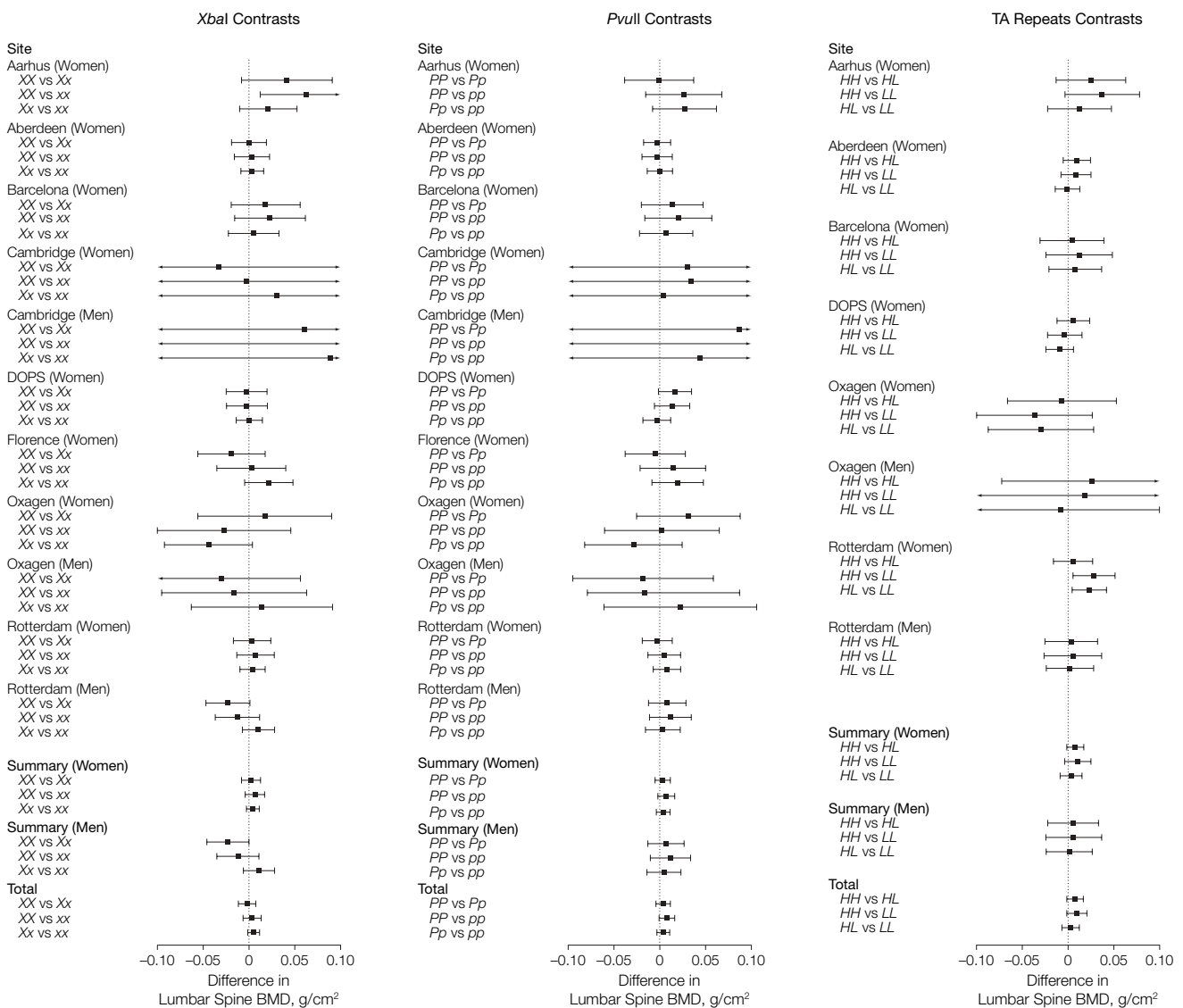
For each study, the point estimates and 95% confidence intervals for the differences in BMD are illustrated. Syntheses were obtained with random-effects analysis. Fixed-effects estimates are similar (not shown). DOPS indicates Danish Osteoporosis Study.

volved a higher BMD, with *XX* over *Xx* and *xx* and with *PP* over *Pp* and *pp*, but differences were small and nonsignificant. Maximum differences for marginal means were less than 0.01 g/cm² overall and for women.

Results were similar using haplotypes (not shown in detail). The adjusted differences in BMD between all genetic contrasts in women were always 0.01 g/cm² or less at either skeletal site (with maximal trends typically show-

ing a higher BMD in E haplotype homozygotes). No clear differences were observed in men. Interactions of age or menopausal status with genotype were not statistically significant for any of these analyses (data not shown).

Fracture Risk

Genotype Analyses. For recessive and dominant models of genetic contrasts (**TABLE 2**), there was no statistically significant between-study heterogeneity

for any of the comparisons either for all fractures or for vertebral fractures alone (heterogeneity *P* > .10). Thus, fixed- and random-effects results were similar, although in a few cases random effects were somewhat more conservative in terms of the level of statistical significance (Table 2).

There was a highly significant protection conferred by the *XX* genotype against the overall fracture risk, with approximately 20% reduction in the odds

Figure 3. Differences in Femoral Neck Bone Mineral Density (BMD) for the Contrasts of *Xba*I, *Pvu*II, and TA Repeat Genotypes
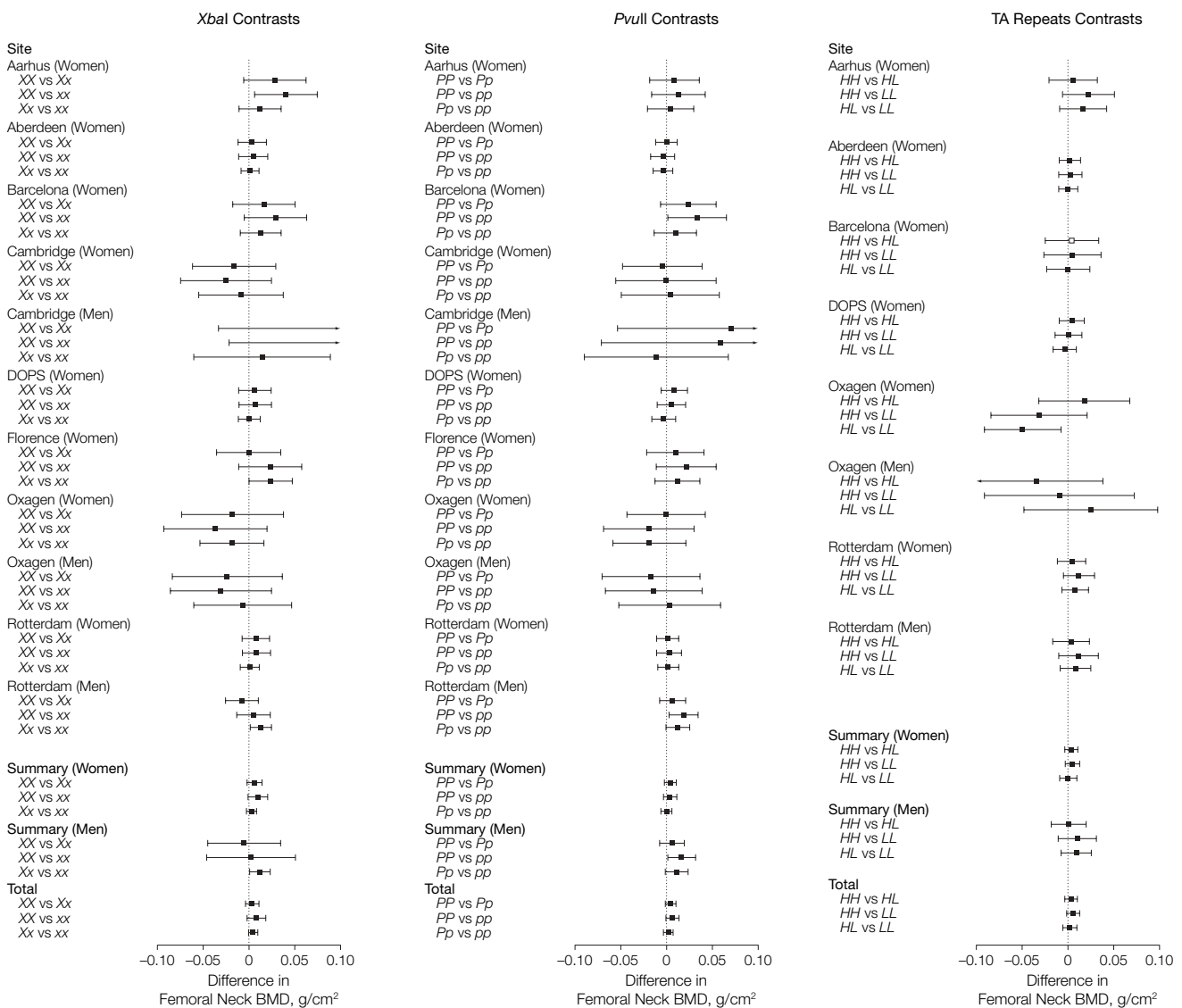
For each study, the point estimates and 95% confidence intervals for the differences in BMD are illustrated. Syntheses were obtained with random-effects analysis. Fixed-effects estimates are similar (not shown). DOPS indicates Danish Osteoporosis Study.

(**Figure** 4, *P*<.001 by both fixed and random effects), and the magnitude of the effect was similar in women and men. The risk for individuals with the *Xx* genotype did not differ from those with *xx* (fixed-effects OR, 1.02; 95% confidence interval [CI], 0.94-1.10), consistent with a recessive effect of *XX* on fracture risk. A favorable trend with *PP* disappeared when analyses excluded *XX* homozygotes (fixed-effects OR, 1.03; 95% CI, 0.91-1.17). TA repeats showed no effect.

For vertebral fractures, there was an approximately 30% reduction in the odds of fractures with *XX* (Figure 4, *P*<.001 by fixed effects and *P*=.02 by random effects) and no difference in the fracture risk between *Xx* and *xx* (fixed-effects OR, 1.11; 95% CI, 0.96-1.28); dominant models also showed significant protective effects in the absence of *xx, pp* or *LL* (Table 2). Results were largely consistent for women and men.

Haplotype Analyses. The results of haplotype contrasts (**Table** 3) were consistent with the results of genotype contrasts. There was no significant between-study heterogeneity for any of the analyses (heterogeneity *P*>.10). When all fractures were considered, there was 20% reduction in the odds of fractures in women homozygous for the E haplotype (Figure 4). For vertebral fractures, 30% to 50% odds reductions were observed with either homozygosity for E haplotype or lack of homozygosity for the A haplotype (Figure 4), and differences between these 2 genetic models were subtle.

Sensitivity and Adjusted Analyses

Analyses limited to incident fractures suggested a similar effect for *XX* in women: OR, 0.83; 95% CI, 0.68-1.01; *P*=.07 for any incident fracture (OR, 0.77; *P*=.04 for the Rotterdam cohort, in which a systematic effort was made to record radiographically vertebral fractures) and 0.54 (95% CI, 0.26-1.13) for radiographically screened incident vertebral fractures (Rotterdam). Data on men (from the Rotterdam cohort) showed no effect, but they

were limited and thus inconclusive (OR, 0.99; 95% CI, 0.65-1.51 and 0.89; 95% CI, 0.38-2.09, respectively, for any fracture and vertebral fractures).

Analyses limited to no-trauma/low-energy-trauma fractures suggested a similar effect for *XX* in women (OR, 0.74; 95% CI, 0.61-0.90; *P*=.002 by fixed effects and OR, 0.79; 95% CI, 0.60-1.03; *P*=.08 by random effects, with no significant between-study heterogeneity). Data on men showed no effect, but they were limited and thus inconclusive (OR, 1.01 by fixed and random effects).

Analyses excluding women who had received any hormone therapy also showed a strong protective effect for *XX* both for any fracture (OR, 0.71; 95% CI, 0.61-0.84; *P*<.001, with no between-study heterogeneity) and for vertebral fractures (OR, 0.60; 95% CI, 0.45-0.82; *P*=.001 by fixed effects and OR, 0.65; 95% CI, 0.46-0.92; *P*=.002 by ran-

dom effects, with no significant between-study heterogeneity).

After adjustment for age, height, weight, and menopausal status, the OR for any fractures in women and men with the *XX* genotype vs *Xx* and *xx* was 0.81 (95% CI, 0.71-0.93; *P*=.002) and 0.91 (95% CI, 0.70-1.18; *P*=.48), respectively. The respective adjusted ORs for vertebral fractures were 0.65 (95% CI, 0.49-0.87; *P*=.003) and 0.84 (95% CI, 0.51-1.37; *P*=.48). After further adjustment for BMD values, the estimates remained largely unchanged. For example, for women the OR for any fractures remained 0.81 (95% CI, 0.71-0.93; *P*=.003) after adjustment for lumbar spine BMD and 0.83 (95% CI, 0.72-0.95; *P*=.006) after adjustment for femoral neck BMD, whereas after adjustment for lumbar spine BMD, the OR for vertebral fractures became 0.61 (95% CI, 0.45-0.82; *P*=.001). With fur-

Table 2. Fracture Risk in Recessive- and Dominant-Model Genetic Contrasts for Each Polymorphism*

Genotype Contrast	Odds Ratio (95% Confidence Interval)			
	Recessive Model, Fixed Effects	Recessive Model, Random Effects	Dominant Model, Fixed Effects	Dominant Model, Random Effects
Any fracture site				
*Xba*I				
All	0.79 (0.70-0.90)	0.80 (0.70-0.91)	0.93 (0.86-1.01)	0.93 (0.86-1.01)
Men	0.83 (0.60-1.15)	0.83 (0.60-1.16)	0.90 (0.73-1.11)	0.90 (0.73-1.10)
Women	0.79 (0.69-0.90)	0.79 (0.67-0.94)	0.94 (0.86-1.02)	0.94 (0.86-1.02)
*Pvu*II				
All	0.90 (0.81-0.99)	0.90 (0.82-0.99)	0.95 (0.87-1.04)	0.95 (0.87-1.03)
Men	0.85 (0.66-1.10)	0.85 (0.66-1.10)	0.91 (0.73-1.13)	0.91 (0.73-1.13)
Women	0.91 (0.82-1.01)	0.91 (0.82-1.01)	0.96 (0.87-1.05)	0.94 (0.84-1.05)
TA repeats				
All	0.94 (0.84-1.06)	0.95 (0.84-1.06)	0.93 (0.84-1.03)	0.93 (0.84-1.03)
Men	0.94 (0.67-1.32)	0.94 (0.67-1.32)	0.83 (0.61-1.11)	0.83 (0.61-1.11)
Women	0.95 (0.84-1.07)	0.94 (0.83-1.07)	0.94 (0.84-1.05)	0.94 (0.84-1.05)
Vertebral fracture				
*Xba*I				
All	0.67 (0.52-0.86)	0.71 (0.53-0.94)	0.83 (0.72-0.96)	0.83 (0.71-0.98)
Men	0.75 (0.43-1.31)	0.75 (0.43-1.31)	0.96 (0.69-1.32)	1.22 (0.61-2.45)
Women	0.65 (0.49-0.86)	0.72 (0.48-1.08)	0.80 (0.68-0.94)	0.79 (0.67-0.94)
*Pvu*II				
All	0.81 (0.67-0.98)	0.85 (0.69-1.07)	0.79 (0.68-0.93)	0.79 (0.67-0.92)
Men	0.78 (0.51-1.18)	0.79 (0.52-1.20)	0.81 (0.58-1.13)	0.87 (0.54-1.41)
Women	0.82 (0.66-1.01)	0.90 (0.68-1.19)	0.79 (0.66-0.95)	0.79 (0.66-0.94)
TA repeats				
All	0.73 (0.57-0.94)	0.75 (0.59-0.97)	0.70 (0.57-0.86)	0.70 (0.57-0.86)
Men	0.66 (0.35-1.22)	0.69 (0.38-1.28)	0.62 (0.39-0.99)	0.62 (0.39-0.99)
Women	0.75 (0.57-0.99)	0.76 (0.58-1.01)	0.72 (0.58-0.91)	0.72 (0.57-0.91)

*Recessive model contrasts: *XX* vs *Xx* and *xx*, *PP* vs *Pp* and *pp*, *HH* vs *HL* and *LL*. Dominant model contrasts: *XX* and *Xx* vs *xx*, *PP* and *Pp* vs *pp*, *HH* and *HL* vs *LL*.

ther adjustment for physical activity and ability indices in the 5 cohorts with available data, the results remained similar. For example, for women the OR for any fractures remained 0.78 (95% CI, 0.67-0.91), and the OR for vertebral fractures was 0.72 (95% CI, 0.46-1.14). Other adjusted estimates were also similar to the unadjusted results, and there was no significant interaction between genotype and age or menopausal status (not shown).

COMMENT

In this multicenter study including individual-level information from almost 20 000 individuals, we found that the *ESR1* gene exerts differential genetic effects on BMD and fracture risk. Effects on BMD were either absent or of small magnitude, whereas there was a statistically significant, 20% reduction in the odds of fractures and a possibly even larger protective effect against vertebral fractures in *XX* homozygous individuals.

Although we did not standardize BMD measurements across all centers, the method of data analysis was based on genotype-related differences in BMD

Figure 4. Odds Ratio for Fractures for the Contrasts of Genotypes and Long-Range Haplotypes
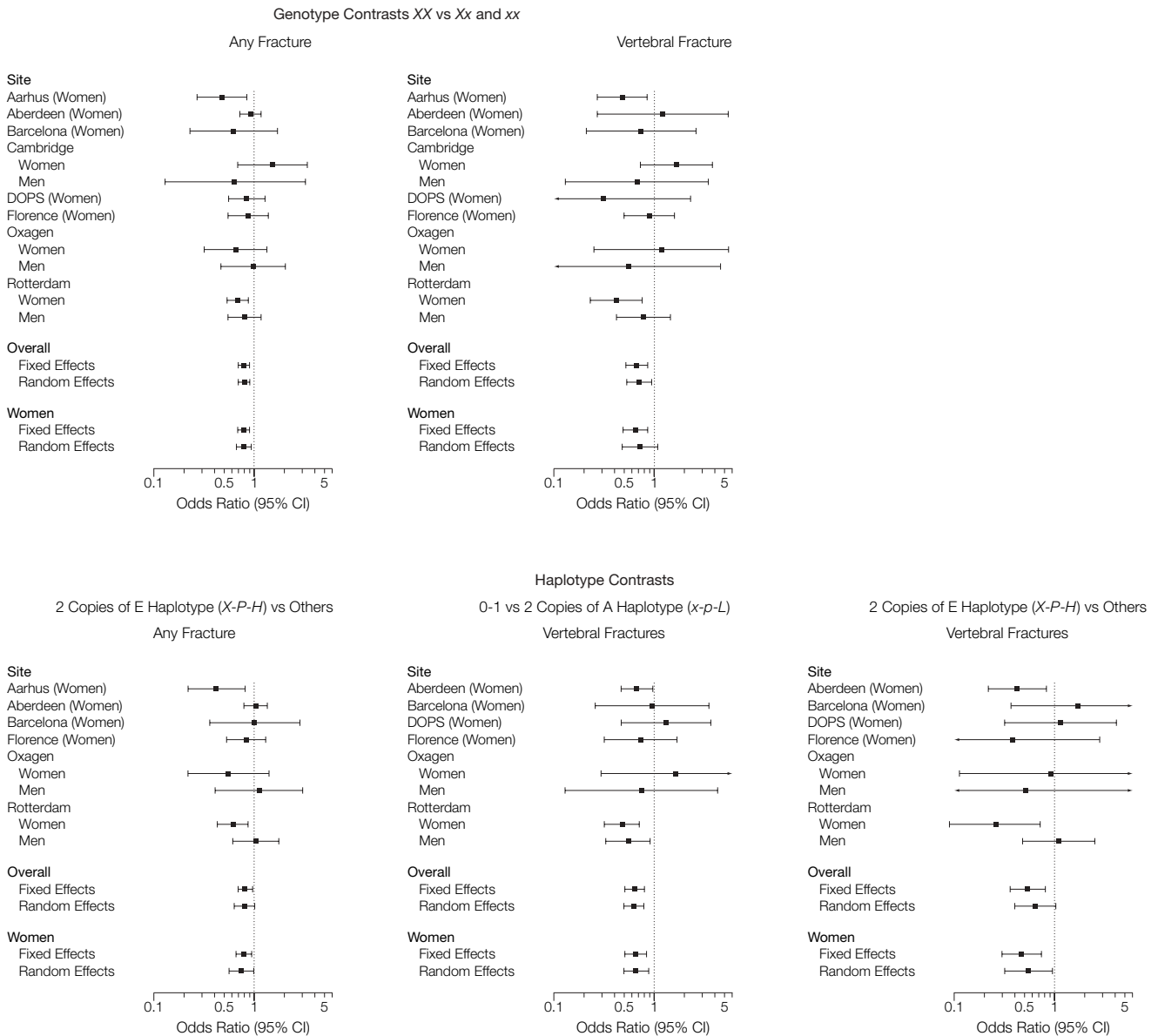
Point estimates and 95% confidence intervals (CIs) are shown for the odds ratio in each study. At the bottom of the graphs, summary estimates of the odds ratios and their 95% CIs are given by fixed-effects and random-effects models for the total database and for women only.

within each center to circumvent between-center differences in the type or model of densitometer used. In any case, the observed 20% to 40% reduction in the risk of fractures we observed would correspond to BMD differences of 0.030 to 0.080 g/cm² in epidemiologic cohorts,[19] which should have been easily detectable, given the sample size of almost 20 000 individuals studied here. Nonetheless, clinical trials of osteoporosis treatments have suggested that fracture risk reduction may be disproportionately large compared with the corresponding changes in BMD.[20,21] For example, in the Fracture Intervention Trial, changes in spine BMD explained only 16% of the reduction in the risk of vertebral fracture with alendronate.[22] Our findings are consistent with the hypothesis that the *Xba*I polymorphism influences fracture risk independent of BMD, even though BMD would have been a plausible biological mediator of the clinical effect for polymorphisms involved in the estrogen pathway. Possibilities include effects on bone quality, bone geometry, bone turnover, or other nonskeletal risk factors for fracture, such as decreased cognition or muscle strength. These candidate mediators need to be better studied, and there is a rapidly increasing literature on pleiotropic actions of *ESR1* on various outcomes.[23-26] Whatever the mechanism, the observed association has potential clinical relevance because it indicates that genotyping for the *ESR1 Xba*I polymorphism provides information on fracture risk that cannot be obtained by BMD measurements alone.

From a methodologic point of view, our study had the advantage of using individual participant data to allow consistent standardization of definitions, measurements, and genetic contrasts. Sampling and systematic errors are a threat to molecular studies.[27] We ensured the consistency and reliability of genotype results across the participating cohorts. Eventually, the results from all the diverse cohorts included in our consortium were similar, and there was no significant between-study heterogeneity detected in any of the analyses of

Table 3. Fracture Risk in Contrasts Involving Combinations of Haplotypes

Subjects by Genotype Contrast	Fixed Effects OR (95% CI)	Random Effects OR (95% CI)
Any fracture site		
Others vs 2 copies of *x-p-L* (A haplotype)		
All	0.89 (0.79-0.99)	0.88 (0.78-0.99)
Men	0.84 (0.61-1.16)	0.84 (0.61-1.16)
Women	0.90 (0.79-1.01)	0.87 (0.74-1.03)
2 Copies of *X-P-H* (E haplotype) vs others		
All	0.81 (0.69-0.96)	0.80 (0.63-1.01)
Men	1.05 (0.66-1.67)	1.05 (0.65-1.67)
Women	0.79 (0.66-0.94)	0.74 (0.55-0.99)
Vertebral		
Others vs 2 copies of *x-p-L* (A haplotype)		
All	0.63 (0.50-0.78)	0.62 (0.49-0.77)
Men	0.57 (0.35-0.91)	0.56 (0.35-0.91)
Women	0.64 (0.50-0.83)	0.65 (0.49-0.88)
2 Copies of *X-P-H* (E haplotype) vs others		
All	0.54 (0.36-0.80)	0.64 (0.39-1.03)
Men	0.96 (0.43-2.19)	1.04 (0.47-2.31)
Women	0.46 (0.29-0.73)	0.55 (0.31-0.97)

Abbreviations: CI, confidence interval; OR, odds ratio.

interest. Between-study heterogeneity is observed in about half the cases in which different teams publish data on the same putative gene-disease association.[10,13] Sometimes this heterogeneity may be due to technical differences and lack of standardization across different centers rather than to genuine genetic diversity. Furthermore, although our consortium design does not accommodate all previously published data, these are limited[3] compared with the evidence that we generated. A meta-analysis in which genotyping is performed prospectively is immune to the problems of publication bias[28] because all prospective, standardized genotyping results are eventually included in the analysis and inclusion is not determined by the direction or strength of the findings. Publication bias against studies that find no significant association may be a problem in genetic association studies[13] and may be another reason for the occurrence of variability among the results of studies published in the literature.

The *Xba*I and *Pvu*II polymorphic sites are located in the first intron of the *ESR1* gene, and so far their functional consequences are unknown. However, introns may contain regulatory elements. For example, the *Pvu*II polymorphism is located within a potential bMyb binding site with regulatory effects on

a reporter gene.[29] In the absence of definitive evidence for the functionality of these *ESR1* variants, more research is needed on the potential biological pathways that they may affect. Alternatively, other polymorphic sites in strong linkage disequilibrium with those that we studied may be functional variants affecting receptor structure or, more likely, messenger RNA and protein expression. A comprehensive analysis of the *ESR1* gene might require the genotyping of a large number of gene variants. However, it is impractical to perform meta-analyses of such large scale on an extended number of unselected polymorphisms; targets for obtaining large-scale genetic evidence should be selected carefully according to preliminary smaller studies, as in this case. Osteoporosis risk may also be modulated by a large number of genetic markers beyond *ESR1*, including polymorphisms of the vitamin D receptor (*VDR*) gene,[30] the collagen I α₁ (*COLIA1*) gene,[31] and several other candidate genes.[2] Although the clinical impact of each implicated gene polymorphism is modest, the cumulative effect may be large. Moreover, clarification of the role of these genetic variants with large-scale evidence may give us important biological insights, such as the extent to which effects on fractures diverge from BMD effects.

The current meta-analysis emphasizes the need for large-scale studies to clarify postulated genetic determinants of osteoporosis and other complex multigenetic diseases.[12,13] Meta-analyses of individual-level data in other fields have also suggested that plausible genetic associations may be refuted with larger-scale evidence[32,33] or may be partially replicated.[34] Quantifying genetic risks for fractures and other osteoporosis outcomes will require adequately powered studies, standardization, and relevant disease end points.

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