Annals of Internal Medicine | ARTICLE

The Association between Common Vitamin D Receptor Gene Variations and Osteoporosis: A Participant-Level Meta-Analysis

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Background: Polymorphisms of the vitamin D receptor (*VDR*) gene have been implicated in the genetic regulation of bone mineral density (BMD). However, the clinical impact of these variants remains unclear.

Objective: To evaluate the relation between *VDR* polymorphisms, BMD, and fractures.

Design: Prospective multicenter large-scale association study.

Setting: The Genetic Markers for Osteoporosis consortium, involving 9 European research teams.

Participants: 26 242 participants (18 405 women).

Measurements: Cdx2 promoter, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms; BMD at the femoral neck and the lumbar spine by dual x-ray absorptiometry; and fractures.

Results: Comparisons of BMD at the lumbar spine and femoral neck showed nonsignificant differences less than 0.011 g/cm^2 for any genotype with or without adjustments. A total of 6067 participants reported a history of fracture, and 2088 had vertebral fractures. For all *VDR* alleles, odds ratios for fractures were very close to 1.00 (range, 0.98 to 1.02) and collectively the 95% CIs ranged from 0.94 (lowest) to 1.07 (highest). For vertebral fractures, we observed a 9% (95% CI, 0% to 18%; $P = 0.039$) risk reduction for the Cdx2 A-allele (13% risk reduction in a dominant model).

Limitations: The authors analyzed only selected *VDR* polymorphisms. Heterogeneity was detected in some analyses and may reflect some differences in collection of fracture data across cohorts. Not all fractures were related to osteoporosis.

Conclusions: The *Fok*I, *Bsm*I, *Apa*I, and *Taq*I *VDR* polymorphisms are not associated with BMD or with fractures, but the Cdx2 polymorphism may be associated with risk for vertebral fractures.

Ann Intern Med. 2006;145:255-264. www.annals.org

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*For a list of study investigators, see the Appendix.

Osteoporosis is a common health problem, especially in elderly persons. Our ability to predict which patients are most likely to sustain fractures is still limited and incomplete. Bone mineral density (BMD) testing and risk factor assessment still do not accurately identify patients who eventually have fractures. Some of the unknown risk factors may be genetic.

One of the first postulated discoveries in the genetics of complex diseases pertained to osteoporosis (1). One team found that allelic variation in the vitamin D receptor (*VDR*) gene explains 75% of the genetic variability in BMD (1). This was a relatively small study (125 twin pairs and 311 unrelated women) that focused on a G→A change (rs1544410) detected as a *Bsm*I restriction fragment length polymorphism in intron 8. The study was published in *Nature* (1) in 1994. Subsequently, genotyping errors were identified in this study, and the results were modified (1). Yet, the *VDR* gene continued to figure prominently in the genetics of osteoporosis and beyond. The original report has been cited more than 1000 times.

Numerous small studies (typically with <1000 participants, with few exceptions) tried to replicate and extend this observation, but the results were inconsistent. The *Bsm*I polymorphism is in strong linkage disequilibrium with 2 other polymorphisms, detected as *Apa*I (rs17879735) and *Taq*I (rs1788009) restriction fragment length polymorphisms. This means that these polymorphisms coexist far more frequently than by chance; pairwise D' values are 0.95 to 0.98 (1.00 means perfect linkage disequilibrium). Thus, one should consider haplotype analyses including all 3 polymorphisms (1, 2). Three meta-analyses of the available small studies have documented associations of *Bsm*I or *Bsm*I–*Apa*I–*Taq*I haplotypes with BMD. However, these meta-analyses have used disparate methods and selectively reported data. Moreover, summary effects were generally modest (3–5). Investigators have also studied other *VDR*

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Context

Some studies suggest that multiple polymorphisms of the vitamin D receptor (*VDR*) gene influence regulation of bone mineral density (BMD).

Contribution

This multicenter, prospective study involving 26 242 participants examined associations between *VDR* polymorphisms (Cdx2-promoter, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I) and BMD and fractures. These polymorphisms were not associated with lumbar spine or femoral neck BMD. Only the Cdx2 A-allele was associated with reduced risk for vertebral fracture.

Cautions

The study did not examine all *VDR* polymorphisms.

Implications

Contrary to previous claims, the *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms probably do not affect BMD or fracture risk.

—The Editors

polymorphisms. A G→A polymorphism (rs17883968) exists in a Cdx2 binding site in the 1a/1e promoter region (6, 7). A C→T polymorphism (a start codon change) results in a *Fok*I restriction fragment length polymorphism (rs17881966) (8).

These 5 polymorphisms may determine BMD and fracture risk, the clinically pertinent outcomes of osteoporosis, on a population level. Therefore, we performed a large-scale collaborative association study with standardized genotyping and definitions.

METHODS

The Genetic Markers for Osteoporosis (GENOMOS) consortium (9) performed a collaborative analysis that included 26 242 participants enrolled from 9 European teams. The consortium used prospective genotyping with cross-center standardization. Main outcomes were BMD at the lumbar spine and femoral neck, all fractures, and vertebral fractures.

Organizational Issues

The GENOMOS project is a large-scale study of several candidate gene polymorphisms for osteoporosis outcomes (9). Our report includes 7 of the 8 teams included in a different meta-analysis of *ESR1* gene polymorphisms (9). It also includes participants from 3 more teams in Graz, Austria; Amsterdam, the Netherlands; and Poland. We did not include 1 of the previously studied cohorts (the Danish Osteoporosis Study) because of potential coding errors of some samples. Two participating teams (Rotterdam, the Netherlands, and Barcelona, Spain) provided additional fracture data on some participants, 3 teams (European Prospective Osteoporosis Study [EPOS]; Aarhus, Denmark; and Florence, Italy) provided additional fracture and genotype data, and 1 team (Aberdeen, United Kingdom) updated and validated its fracture database compared with our *ESR1* gene analysis (9).

We refer to previous publications regarding the characteristics of 4 longitudinal cohorts: the Rotterdam study (10), the Aberdeen Prospective Osteoporosis Study (11), the Longitudinal Aging Study Amsterdam (12), and EPOS (13). We have also previously described 4 cross-sectional studies (Aarhus; Barcelona; Florence; and Oxagen, Abingdon, United Kingdom) (9, 14). The current investigation also included data from the cross-sectional European Polish Osteoporosis Study (EPOLOS). This study is an affiliate of EPOS and was analyzed in the current study as part of the EPOS database. The EPOLOS is a population-based study in Poland involving white men and women who were 20 to 80 years of age. The exclusion criteria were pregnancy, cancer, obesity (weight >100 kg), and fractures during the year before enrollment. Our analysis included EPOLOS participants with fractures and age- and sex-matched control participants. We also included data from a study coordinated in Graz, Austria; participants were selected from a population-based study of healthy Austrians (21 to 76 years of age) (15) and from a study of elderly nursing home residents (average age, 84 years) in eastern Austria. Only the clinic-based studies (Aarhus, Barcelona, Florence, and Graz) excluded patients with long-term steroid use and primary hyperthyroidism.

All participating teams contributed information on sex, age, height, weight, *VDR* genotype, and BMD (mg/cm^2) at the lumbar spine and femoral neck. Teams also provided information on menopausal status and use of hormone replacement therapy for women and on fractures (any fracture and vertebral fractures). However, fracture information was gathered differently by the participating centers. Therefore, we also separately examined incident fractures, incident vertebral fractures, and low- and no-trauma fractures in sensitivity analyses.

Prevalent Fractures

In the Aarhus, Rotterdam, and Barcelona studies, fractures occurring at or before enrollment had radiographic documentation. In the Graz, Amsterdam, Barcelona, Florence, and Oxagen studies and in EPOS, participants with fractures had clinical history or questionnaire documentation. All participants with vertebral fractures had radiographic documentation with clinical or morphometric criteria (16), except those in the Aberdeen study (questionnaire only). The Amsterdam, Florence, and Oxagen studies counted fractures occurring at any time of life. The Aberdeen study excluded fractures in patients younger than age 18 years, EPOS and the Graz study excluded fractures in patients younger than age 20 years, and the Barcelona study excluded fractures in patients younger than age 45 years. The Aarhus and Rotterdam studies only

counted vertebral fractures documented radiographically at enrollment; the Barcelona study excluded fractures of the hands, fingers, toes, feet, face, and skull; the Graz study excluded fractures of the hands, face, skull, and clavicle; and the Barcelona and Florence studies and EPOS excluded high-trauma fractures.

Incident Fractures

Longitudinal studies also reported available data on incident fractures that had occurred during follow-up. These studies only included fractures validated by medical records, scrutiny of original radiographs, or radiologist reports, except for EPOS (interviewer-completed questionnaire). We had radiographs for all incident vertebral fractures at the time of the clinical presentation.

Genotyping

We genotyped rs17883968 (Cdx2), rs17881966 (*Fok*I), rs1544410 (*Bsm*I), rs17879735 (*Apa*I), and rs17880019 (*Taq*I) by using various techniques: TaqMan (Applied Biosystems, Foster City, California; Aberdeen, Florence, Graz, Oxagen, and Rotterdam studies and EPOS), pyrosequencing (Oxagen study), restriction fragment length methods (Aarhus, Aberdeen, and Barcelona studies), sequencing (Aberdeen study), and SNaPshot Multiplex System (Applied Biosystems; Barcelona study). We cross-validated these different methods by blinded genotyping of 50 randomly selected samples from all centers. The coordinating center in Rotterdam evaluated the results and reported any discrepancies in the reference plate to improve calling of genotypes. We repeated genotyping of the reference plate, and centers had to switch genotyping techniques if they were still generating more than 5% errors. One center changed from using the restriction fragment method to using TaqMan, and another center changed from using sequencing to using the restriction fragment method. In addition, each center checked its own cohort genotyping afterward by reanalyzing at least 5% of its samples with random selection. Discrepancy rates of less than 1% were observed for each center.

Measurements of BMD

We measured BMD by dual-energy x-ray absorptiometry. The Aarhus, Amsterdam, Barcelona, Florence, and Graz studies used Hologic bone densitometers (Hologic, Bedford, Massachusetts); the Aberdeen study used Norland XR26 and XR36 densitometers (Cooper Surgical, Trumbull, Connecticut); the Rotterdam study used Lunar DPX-L or DPX densitometers (GE Medical Systems, Madison, Wisconsin); and EPOS and the Oxagen study used various devices cross-calibrated with the European Spine Phantom (17). Syntheses of BMD data across studies always included a study effect to account for differences between samples and between centers. We interpreted results of the meta-analysis for BMD with emphasis on the BMD differences (absolute differences in the mean values of BMD across genotypes). We did not focus on absolute BMD values because the absolute values may depend on the measuring device.

Outcomes

The main outcomes include BMD at the lumbar spine and femoral neck, all fractures (as defined by each cohort), and vertebral fractures defined by clinical or morphometric criteria (16). We also performed sensitivity analyses for incident fractures, incident vertebral fractures, and low- and no-trauma fractures. The latter excluded high-trauma fractures, as assessed by the circumstances in which they had occurred, their location, or both. All teams except those from Aberdeen and Graz had information on low- and no-trauma fractures.

Participants were unrelated in all studies except for the Familial Osteoporosis Study (FAMOS), which is a family-based study coordinated by the company Oxagen (Abingdon, United Kingdom). For FAMOS, we selected 1 participant per pedigree (using random-number selection in each pedigree). Sensitivity analysis that used all Oxagen participants yielded similar results (not shown).

Polymorphisms and Haplotypes

We analyzed *Cdx2* and *Fok*I as single nucleotide polymorphisms separately and analyzed the other 3 linked polymorphisms as haplotypes. We inferred haplotypes using the PHASE program (Matthew Stephens, University of Washington, Seattle, Washington). The algorithm uses a Bayesian approach and estimates how alleles from different polymorphisms in linkage disequilibrium coexist on the same chromosome. The approach also estimates the uncertainty for each inferred haplotype (18).

Statistical Analyses

For all analyses, we first split data from each participating team according to sex when the team had enrolled men and women. We could not exclude the possibility that genetic effects may be different in men and women. Therefore, the connotation of study level pertains to data from a specific team for a specific sex. The optimum approach for the analysis of these consortium data should consider that the frequency of the genetic markers of interest may vary across different study samples. Therefore, all analyses took study into consideration. Second, we considered the possibility that not only genetic marker frequencies but also genetic effects may vary across studies. Therefore, we treated study as a random effect. When there was no evidence of different genetic effects across different studies, random effects would not have an advantage over fixed effects (or simple stratified analyses); the latter are presented for simplicity. Moreover, we also performed analyses with further adjustments for other covariates that may be important for osteoporosis outcomes. Adjusted analyses were not necessarily given precedence over unadjusted analyses: Unadjusted analyses may be unbiased on the basis of Mendelian randomization, whereas adjusted analyses account for additional factors but may have missing informa-

Table 1. **Differences in Adjusted Bone Mineral Density Values for Vitamin D Receptor Cdx2 and *FokI* Genotype Comparisons***

Sex	Polymorphism	Difference in Estimated Marginal Means of BMD (95% CI), *mg/cm²*			
		Lumbar Spine	*P* Value	Femoral Neck	*P* Value
Women†	Cdx2				
	GG vs. *GA*	3 (−4 to 9)	0.38	3 (−2 to 8)	0.13
	GG vs. *AA*	−8 (−23 to 8)		−9 (−19 to 4)	
	GA vs. *AA*	−10 (−26 to 5)		−11 (−23 to 1)	
	FokI				
	CC vs. *CT*	4 (−3 to 11)	0.19	1 (−4 to 6)	0.76
	CC vs. *TT*	8 (−1 to 18)		−2 (−9 to 5)	
	CT vs. *TT*	4 (−5 to 13)		−3 (−10 to 4)	
Men†	Cdx2				
	GG vs. *GA*	0 (−12 to 12)	0.48	3 (−5 to 11)	0.74
	GG vs. *AA*	−19 (−49 to 12)		−2 (−22 to 19)	
	GA vs. *AA*	−18 (−50 to 13)		−5 (−26 to 16)	
	FokI				
	CC vs. *CT*	2 (−10 to 14)	0.43	−5 (−14 to 3)	0.02
	CC vs. *TT*	11 (−6 to 29)		12 (0 to 24)	
	CT vs. *TT*	9 (−8 to 26)		17 (5 to 29)	

* Values are derived from mixed-effects model (random effects for study and fixed effects for genotype) and adjusted for age, weight, and height for men and adjusted additionally for menopausal status and use of hormone replacement therapy for women. *P* values do not adjust for multiple comparisons. BMD = bone mineral density.
† For all effects, estimates in men vs. women are not significantly different ($P > 0.10$), with the exception of *CT* vs. *TT* for *Fok*I in the femoral neck.

tion for some covariates and can also lead to some unavoidable subjectivity in selection of covariates.

There was no strong biological plausibility that specific genetic models may be more appropriate. Therefore, we decided to use an analysis considering all genotypes (or haplotype pairs) separately for continuous outcomes (BMD) and per-allele analysis for binary outcomes (fractures). In the per-allele model, the relative risk between those carrying 1 copy versus no copies is the same as the relative risk between those carrying 2 copies versus 1 copy of the allele. We also investigated additional models (dominant and recessive) as secondary analyses. The dominant model assumes that the risk changes by carrying at least 1 copy of a specific allele or haplotype. The recessive model assumes that the risk is changed by carrying 2 copies of a specific allele or haplotype. We considered haplotypes separately only if they had a frequency of 15% or higher. Analyses considering all haplotypes yielded similar results (not shown).

Table 2. **Differences in Adjusted Bone Mineral Density Values for Comparisons Involving the Vitamin D Receptor *BsmI–ApaI–TaqI* Haplotype Pairs***

Sex	Haplotype	Difference in Estimated Marginal Means of BMD			
		Lumbar Spine, *mg/cm²*	*P* Value	Femoral Neck, *mg/cm²*	*P* Value
Women†	Pair 11	Reference	0.71	Reference	0.42
	Pair 12	−2		−4	
	Pair 22	6		−5	
	Other	3		−5	
Men†	Pair 11	Reference	0.40	Reference	0.92
	Pair 12	−6		−3	
	Pair 22	7		−1	
	Other	−1		−1	

* The table may be used to calculate all pairwise comparisons (as in Table 1) by subtracting the presented differences. For example, for women the comparison of haplotype pair 22 vs. haplotype pair 12 has a BMD difference of 6−(−2) = 8 mg/cm². Values are derived from mixed-effects model (random effects for study and fixed effects for genotype) and adjusted for age, weight, and height for men and adjusted additionally for menopausal status and use of hormone replacement therapy for women. *P* values take into account all haplotype pairs and do not adjust for multiple comparisons. BMD = bone mineral density.
† For all effects, comparison between women and men shows no statistically significant differences ($P > 0.10$ overall).

For BMD, we performed analysis of variance considering genotypes or haplotype pairs as fixed factors and study as a random factor. This is a mixed-model approach (19). We also performed adjusted analyses by adding covariates to the mixed models. Besides BMD, these analyses included as covariates age, height, weight, and use of hormone replacement therapy and menopausal status for women. For these analyses, we compared estimated marginal means in each genotype or haplotype pair group.

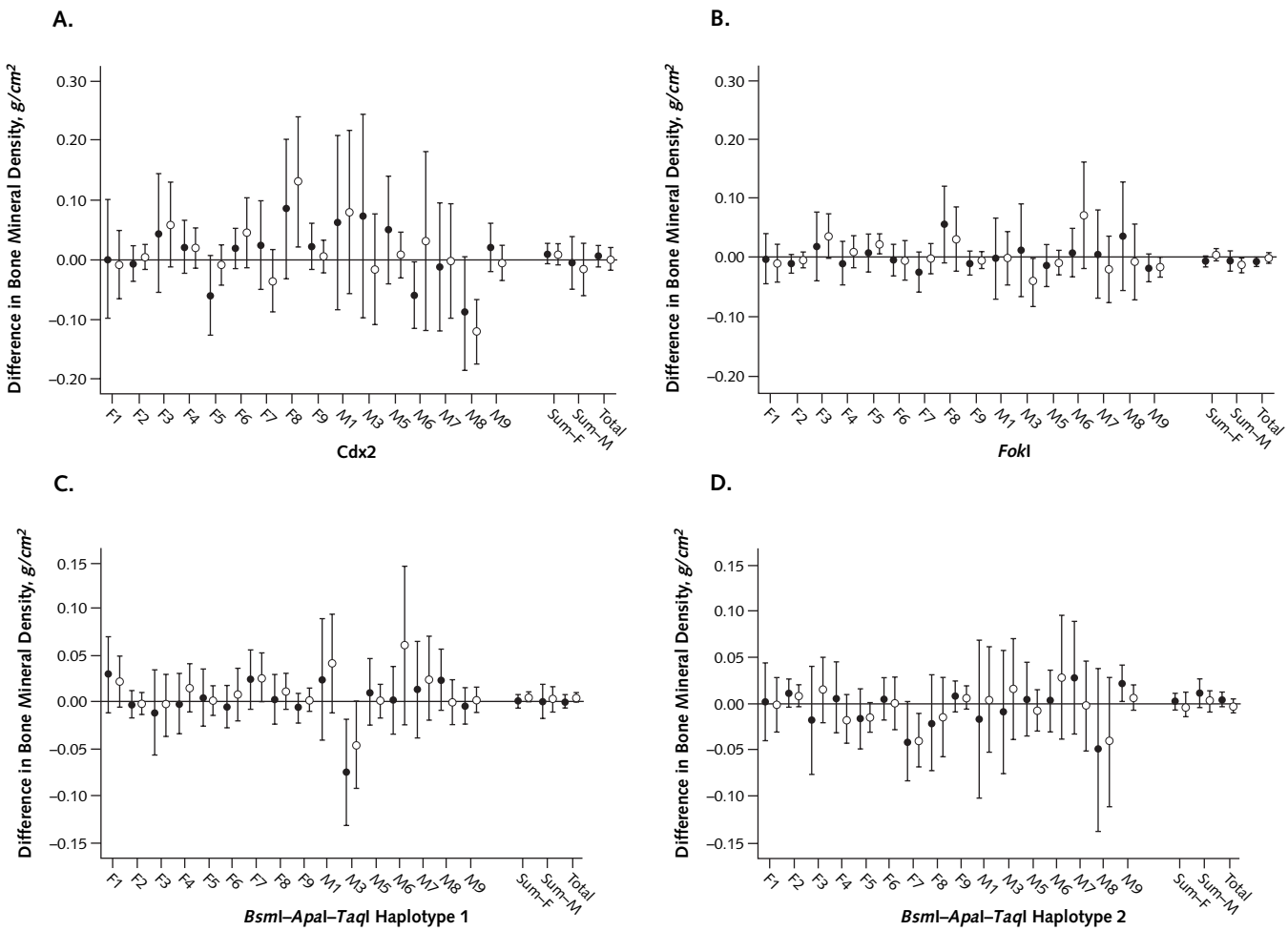
We also estimated the unadjusted mean and standard deviation for BMD in each study for each genotype or haplotype pair of interest. We then synthesized BMD differences between genotype or haplotype pair contrasts across studies using general variance models (inverse variance method) (20). General variance models weigh each study by the inverse of its variance. This variance may or may not take into account the variance between studies (random- and fixed-effects, respectively). We assessed the significance of between-study heterogeneity with the Q statistic (significant for $P < 0.10$). We also reported the I^2 statistic, a metric of the amount of heterogeneity, when there was significant heterogeneity (21). Contrary to the typical meta-analysis of a few small studies in which significance testing for heterogeneity may be underpowered, the studies we analyzed are typically large. Therefore, the test for heterogeneity significance may even be overpowered.

For fractures, we used the odds ratio as the metric of

choice. We synthesized the odds ratios across studies with fixed- and random-effects methods (22). In each analysis, we evaluated odds ratios for between-study heterogeneity using the Q statistic (significant for $P < 0.10$) and reported the I^2 statistic when there was significant heterogeneity (21). We also performed adjusted logistic regression analyses. We stratified these analyses per study and sex and also considered age, height, and weight and use of hormone replacement therapy and menopausal status for women. Age- and study-by-genotype interactions were also considered to test for the possibility of age-dependent and study-dependent genetic effects. Further adjustment for BMD was considered for fracture outcomes when gene variants had statistically significant effects.

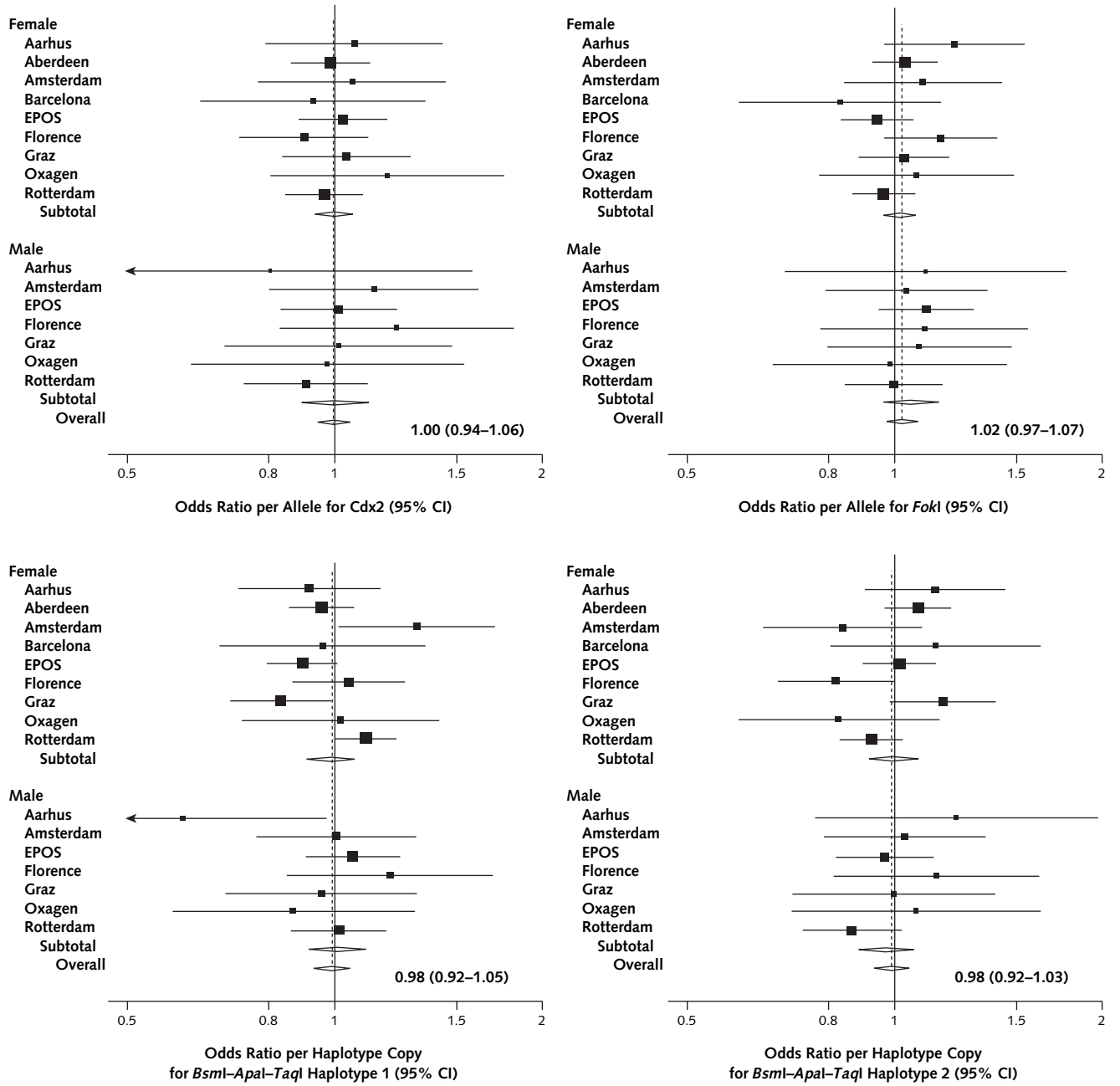
We used SPSS, version 12.0 (SPSS Inc., Chicago, Illinois), and Meta-Analyst (Joseph Lau, Boston, Massachusetts). All reported *P* values are 2-tailed and are not adjusted for multiple comparisons. We performed exact tests for Hardy–Weinberg equilibrium proportions (23) using the GENEPOP program (http://wbiomed.curtin.edu.au/genepop/). This test evaluates whether the distribution of genotypes differs from the expected proportions p^2, $2p(1-p)$, and $(1-p)^2$, where p is the minor allele frequency. All data sets were in Hardy–Weinberg equilibrium, except for those from the Aarhus (for *Fok*I), Graz (for *Bsm*I and *Taq*I), and Rotterdam (for *Bsm*I, *Apa*I, and *Taq*I) studies. Exclusion of these data did not change the summary estimates considerably (not shown).

Figure 1. **Differences in bone mineral density for recessive models for Cdx2 *(A)*, *Fok* I *(B)*, and haplotypes 1 *(C)* and 2 *(D)* of *BsmI–ApaI–TaqI*.**



For each study, the point estimates and 95% CIs for the differences in bone mineral density in the lumbar spine (*solid circles*) and femoral neck (*open circles*) are shown. The study teams are listed alphabetically: 1 = Aarhus; 2 = Aberdeen; 3 = Amsterdam; 4 = Barcelona; 5 = European Prospective Osteoporosis Study (EPOS); 6 = Florence; 7 = Graz; 8 = Oxagen; and 9 = Rotterdam. Summary estimates of the differences and their CIs are given by inverse-variance random-effects models for female (*Sum–F*), male (*Sum–M*), and all participants (*Total*). Fixed-effects estimates are very similar (not shown). There is some between-study heterogeneity in the Cdx2 analysis for bone mineral density at the femoral neck ($P = 0.002$; $I^2 = 54\%$); otherwise no significant between-study heterogeneity is seen for any other comparison and skeletal site. None of the contrasts shown in the total summary exceed 7 mg/cm^2 in bone mineral density differences. The primary analyses considering all genotypes and haplotype frequencies separately are summarized in Table 1 and Table 2. The European Polish Osteoporosis Study (EPOLOS) data are combined with the EPOS data. F = female; M = male.

Figure 2. **Odds ratio for fractures in co-dominant models (per allele) for fracture at any site for Cdx2, *Fok*I, and haplotypes 1 and 2 of *Bsm*I–*Apa*I–*Taq*I.**



Point estimates and 95% CIs are shown for the odds ratio in each study. Summary estimates of the odds ratios and their 95% CIs are given by random-effects (DerSimonian–Laird) models per sex and for the total database. Fixed-effects estimates are very similar (not shown). European Polish Osteoporosis Study (EPOLOS) data are combined with European Prospective Osteoporosis Study (*EPOS*) data.

Ethical Considerations

The ethics committees at all participating centers approved the study per local regulations. Informed consent followed the requirements of each center.

Role of the Funding Source

The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review or approval of the manuscript.

RESULTS

Database

We gathered data on 26 242 participants (18 405 women); 23 926 participants (16 936 women) were ana-

lyzed after selecting only 1 participant for each FAMOS pedigree (**Appendix Table**, available at www.annals.org). Of the 23 926 participants, data on BMD at the lumbar spine, BMD at the femoral neck, all fractures, and vertebral fractures were available for 16 739, 17 133, 23 309, and 18 227 participants, respectively. There were 6067 participants with any fracture, 2088 with vertebral fractures, 2407 with incident fractures (412 had incident vertebral fractures), and 3743 with low- or no-trauma fractures. Genotype and haplotype frequencies were similar across the 9 participating teams (**Appendix Table**, available at www.annals.org).

Analyses of BMD

In unadjusted analysis of variance, all pairwise comparisons considering all data for BMD in both skeletal sites showed nonsignificant differences. All point estimates were less than 11 mg/cm^2 for Cdx2 and *Fok*I genotypes (not shown in detail). Results were consistent for women and men and after adjustment for age, height, weight, and use of hormone replacement therapy and menopausal status for women (**Table 1**). For sex subgroups and adjusted analyses, the maximal difference was 19 mg/cm^2 and 17 mg/cm^2, respectively. Pairwise comparisons of the 2 most common *Bsm*I–*Apa*I–*Taq*I haplotypes (haplotypes 1 and 2) showed that differences were consistently statistically nonsignificant. The point estimates for these haplotypes were always less than 10 mg/cm^2 for all unadjusted analyses overall (not shown) and smaller than 7 mg/cm^2 for all adjusted analyses for both sexes (**Table 2**). The BMD differences in the original *Nature* paper were 10- to 100-fold higher (1). In recessive models (**Figure 1**), all 95% CIs of the overall and subgroup summary estimates did not extend beyond a difference of 24 mg/cm^2 for any of the polymorphisms. The dominant models yielded similar results (CIs of the overall and subgroup summary estimates did not extend beyond 20 mg/cm^2).

Fracture Analyses

For alleles of interest, the point estimates of the odds ratios for all fractures for men and women combined were very close to 1.00 (range, 0.98 to 1.02) (**Figure 2** and **Table 3**). The CIs excluded 7% differences in the odds of fractures between alleles. Similarly, for vertebral fractures, the CIs excluded 11% differences in the odds of fractures for all alleles with the exception of the Cdx2 polymorphism. For this polymorphism, we observed a borderline significant 9% decrease (CI, 0% to 18%; $P = 0.039$ without adjustment for multiple comparisons) in the odds of fractures with the A-allele.

When sensitivity analyses addressed only incident fractures (data from the 4 longitudinal cohorts), odds ratios still ranged from 0.98 to 1.00. When sensitivity analyses addressed only low- and no-trauma fractures (data from 8 studies), odds ratios still ranged from 0.96 to 1.03. For the sensitivity analysis on incident vertebral fractures, only the Cdx2 A-allele showed a possible protective effect (17% decrease in the odds); however, this was not statistically significant ($P = 0.069$) (**Table 3**).

Adjustment for age (and for sex, height, and weight) did not change any of the summary estimates for fracture risk. Interaction terms between age or study and genotype were not formally statistically significant for any of the main outcomes and did not improve model fit. For example, the summary-adjusted odds ratios for all fractures for men and women combined were 1.00 (CI, 0.94 to 1.06),

Table 3. **Random-Effects Analysis for Fracture Risk by Vitamin D Receptor Genotype per Allele Model***

Variable	Participants	Odds Ratio (95% CI)				
		All Fractures	Vertebral Fractures	Incident Fractures	Incident Vertebral Fractures	Low-Trauma and No-Trauma Fractures
Cdx2 allele A	All	1.00 (0.94–1.06)	0.91 (0.82–1.00)	0.98 (0.90–1.07)	0.83 (0.68–1.01)	1.01 (0.95–1.08)
	Women	1.00 (0.93–1.07)	0.94 (0.84–1.04)	0.97 (0.88–1.07)	0.84 (0.67–1.06)	1.00 (0.92–1.08)
	Men	1.01 (0.90–1.13)	0.83 (0.69–0.99)	1.01 (0.84–1.22)	0.80 (0.54–1.18)	1.05 (0.92–1.19)
*Fok*I allele T	All	1.02 (0.97–1.07)	1.03 (0.96–1.11)	1.00 (0.93–1.07)	1.05 (0.90–1.20)	1.03 (0.97–1.09)
	Women	1.01 (0.95–1.06)	1.01 (0.91–1.13)	1.00 (0.92–1.08)	1.01 (0.84–1.21)	1.05 (0.94–1.16)
	Men	1.06 (0.96–1.16)	1.10 (0.96–1.26)	1.01 (0.79–1.29)	1.17 (0.88–1.57)	1.04 (0.94–1.16)
*Bsm*I–*Apa*I–*Taq*I						
Haplotype 1	All	0.98 (0.92–1.05)†	0.99 (0.90–1.10)†	0.99 (0.88–1.10)†	1.05 (0.90–1.22)	1.03 (0.96–1.10)
	Women	0.98 (0.89–1.07)†	1.01 (0.90–1.13)	1.00 (0.88–1.14)	1.08 (0.91–1.28)	1.02 (0.94–1.12)
	Men	1.00 (0.91–1.10)	0.94 (0.76–1.16)	0.95 (0.70–1.28)†	0.97 (0.72–1.30)	1.04 (0.92–1.17)
Haplotype 2	All	0.98 (0.92–1.03)	1.01 (0.93–1.08)	1.00 (0.89–1.12)	0.95 (0.81–1.10)	0.96 (0.91–1.02)
	Women	0.97 (0.90–1.05)†	0.98 (0.89–1.09)	0.98 (0.91–1.06)	0.93 (0.78–1.11)	0.96 (0.88–1.04)
	Men	0.98 (0.89–1.07)	1.05 (0.91–1.22)	1.05 (0.72–1.53)†	0.99 (0.73–1.33)	0.97 (0.88–1.08)

* Values are calculated by using the DerSimonian–Laird method. Data from all 9 teams are included for all fractures and for vertebral fractures; data for incident fractures are derived from 4 teams, and data for low- and no-trauma fractures are derived from 7 teams (see Methods for details). There is no adjustment for covariates.
† Significant between-study heterogeneity with $0.10 > P > 0.01$; between-study heterogeneity ($P < 0.01$) was not seen for any comparison, and comparison of summary estimates for women and men always showed nonsignificant differences ($P > 0.10$ overall). In the presence of significant between-study heterogeneity, I^2 was 40%, 33%, and 53% for all fractures, vertebral fractures, and incident fractures, respectively, with haplotype 1; I^2 was 57% within the subgroup of women for all fractures and 67% within the subgroup of men for incident fractures; with haplotype 2, I^2 was 41% within the subgroup of women for all fractures and 79% within the subgroup of men for incident fractures.

Table 4. **Random-Effects Analysis for Fracture Risk by Vitamin D Receptor Cdx2 Genotype***

Inheritance Model	Participants	Odds Ratio (95% CI)	
		Vertebral Fractures	Incident Vertebral Fractures
Recessive	All	1.13 (0.86–1.49)	1.12 (0.66–1.89)
	Women	1.20 (0.89–1.63)	0.93 (0.49–1.78)
	Men	0.89 (0.48–1.65)	1.60 (0.64–4.03)
Dominant	All	0.87 (0.78–0.97)	0.78 (0.62–0.98)
	Women	0.89 (0.78–1.01)	0.81 (0.62–1.05)
	Men	0.82 (0.66–1.01)	0.72 (0.45–1.13)

* Values were calculated by using the DerSimonian–Laird method. There is no adjustment for covariates. Statistical significance is reached in the dominant model for all participants combined ($P = 0.011$ for vertebral fractures and $P = 0.037$ for incident vertebral fractures, unadjusted for multiple comparisons). There is no significant between-study heterogeneity in any of these analyses ($P > 0.10$ for heterogeneity), and comparison of summary estimates for women and men always showed nonsignificant differences ($P > 0.10$ overall).

1.03 (CI, 0.98 to 1.09), 1.00 (CI, 0.95 to 1.05), and 1.02 (CI, 0.97 to 1.07) for Cdx2, *Fok*I, haplotype 1, and haplotype 2, respectively (per allele). Similar adjusted results were also obtained for women and men and for other fracture outcomes. Different teams used different scales for physical activity or ability; however, genetic effects were similar when adjusted for physical activity or ability per team.

When all 5 polymorphisms were considered in a multivariable model, the odds ratios per allele for fractures were 1.01 (CI, 0.97 to 1.06) for haplotype 1, 1.00 (CI, 0.94 to 1.06) for Cdx2, and 1.03 (CI, 0.98 to 1.08) for *Fok*I. Multivariate results were similar for other fracture outcomes (not shown). Because the *ESR1 XX* genotype has been documented to be important for determining fracture risk in this cohort (9), we included *XX* in the model. The respective odds ratios became 1.00 (CI, 0.94 to 1.06), 0.97 (CI, 0.90 to 1.05), and 1.02 (CI, 0.96 to 1.09). The *ESR1 XX* effect remained formally statistically significant (multivariate adjusted odds ratio, 0.80 [CI, 0.70 to 0.92]).

We observed an association between the Cdx2 polymorphism and vertebral fracture risk in dominant modeling, with a 13% reduction in the odds of vertebral fractures ($P = 0.011$) (**Appendix Figure**, available at www.annals.org). There was a 22% odds reduction in the sensitivity analysis for incident vertebral fractures ($P = 0.037$) (**Table 4**). The results were similar in adjusted analyses (14% and 21% reduction in the odds; $P = 0.010$ and $P = 0.048$, respectively). However, there are 4 main genetic variants (2 polymorphisms and 2 haplotypes), 4 primary outcomes, and several inheritance models. Adjustment for multiple comparisons would invalidate the statistical significance of these modest effects.

DISCUSSION

Overall, we have obtained large-scale evidence that the *Fok*I polymorphism and the *Bsm*I–*Apa*I–*Taq*I haplotypes have no effect on either BMD or fracture risk. This contradicts previous claims that these polymorphisms act as genetic determinants of BMD. The very large sample size allows for tight CIs that exclude even small differences between genotypes and haplotype pairs. We obtained some evidence for a modest effect of the Cdx2 polymorphism on risk for vertebral fracture.

The possible Cdx2 effect is small. Although we followed a predetermined analysis plan, this may still represent a chance finding, because of the very large number of analyses. Previous studies have indicated that the *VDR* Cdx2 polymorphism is a functional variant that influences DNA protein binding and gene transcription (6, 24). Other functional polymorphisms in the *VDR* promoter are in linkage disequilibrium with Cdx2 (24). However, epidemiologic and functional data provide independent lines of evidence. A polymorphism with functional support does not necessarily have clinical importance, especially for a particular disease (25). A very large number of polymorphisms in the human genome probably will have functional effects on the molecular level, but few have been tested and have been found to be related to specific outcomes for each disease.

The *VDR Bsm*I–*Apa*I–*Taq*I polymorphisms provide a paradigm of sequential changes in evidence. For these polymorphisms, there have been early exaggerated claims, documented genotyping error, and significant but downsized genetic effects in meta-analyses of small studies. We found no evidence of association with BMD or fracture in our study. Still, we cannot exclude that *VDR* polymorphisms other than those we tested might play a role in osteoporosis. A more extensive molecular analysis of multiple *VDR* polymorphisms in the Rotterdam study (24) suggests a modest association of haplotypes in the 3′ untranslated region and fracture risk. The association with fracture is in the totally opposite direction from the association with BMD reported in the *Nature* article (1) and the association based on other polymorphisms. These associated haplotypes, however, largely overlap with those defined by *Bsm*I–*Apa*I–*Taq*I alone. The Rotterdam study also showed an association between fractures and *Bsm*I–*Apa*I–*Taq*I in our collaborative analysis, but this was not seen in the overall cumulative results of all studies combined. Therefore, although we cannot exclude the possibility that other 3′ *VDR* polymorphisms contribute to fracture risk, the association may also be a chance finding in a single large cohort.

The strengths of our study include the very large sample size, international collaboration, and lack of publication bias within the consortium. Moreover, we focused on validation of genotyping to minimize genotyping errors and tried to standardize definitions for the outcomes.

Our study also has limitations. Recording and ascertainment of fractures differed across participating teams, which could introduce some unavoidable heterogeneity. It is also possible that many of the fractures were not osteoporotic but were due to other factors, such as trauma. Therefore, we also performed sensitivity analyses that tar-

geted only incident fractures, incident vertebral fractures, and low- and no-trauma fractures. Reassuringly, we found similar results. Another potential limitation is due to missing data in some cohorts. In particular, not all participants had BMD measurements. However, participants and their physicians made the decision to measure BMD without any knowledge of genotype data.

Results were similar in unadjusted and adjusted analyses. Many other variables may affect the risk for fractures. However, given the principle of Mendelian randomization (26), one expects that exposure to these risk factors is similar in persons with different *VDR* genotypes.

The GENOMOS consortium continues to investigate additional genes in osteoporosis outcomes, including the collagen IA1 Sp1 (27) and transforming growth factor-β polymorphisms. The list of candidate genes is increasing rapidly, especially with the advent of discovery-oriented approaches with massive polymorphism testing. We are also expanding the consortium to include additional international teams working on osteoporosis genetics.

Our paper refutes some widely cited claims in the rapidly expanding human genome epidemiology of complex diseases (28, 29). Several findings in human genetics may result from the interplay of chance and selective reporting of "positive" results from relatively small studies and other biases or errors (30–34). Consortia with standardized genotyping, delineated plans of analysis, and standardized phenotype measurements should become more widely implemented in human genome epidemiology. Claims of large genetic effects should be interpreted with caution.

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Grant Support: By the European Commission (grant QLK6-CT-2002-02629). EPOS was financially supported by a European Union Concerted Action Grant under Biomed-1 (BMH1CT920182) and European Union grants C1PDCT925102, ERBC1PDCT 930105, and 940229; the central coordination was also supported by the UK Arthritis Research Campaign, the Medical Research Council (G9321536), and the European Foundation for Osteoporosis and Bone Disease. The European Union's PECO program linked to BIOMED 1 funded in part the participation of the Budapest, Prague, Piestany, Szczecin, and Moscow centers; data collection from Zagreb was supported by a grant from the Wellcome Trust, and the central radiograph evaluation was generously sponsored by the Bundesministerium fur Forschung and Technologie, Germany. The remaining funding was provided by or through the following centers: *Radiological Evaluation*: Department of Radiology and Nuclear Medicine, Free University, Berlin, Germany (D. Felsenberg, W. Gowin, G. Armbrecht); *Participating Investigative Centers*: Institute of Rheumatology, Moscow, Russia (L.I. Benevolenskaya); Royal National Hospital for Rheumatic Diseases, Bath, United Kingdom (A. Bhalla); Hospital de Angra do Herismo, Azores, Portugal (J. Bruges Armas); Asturias General Hospital, Oviedo, Spain (J.B. Cannata Andia and M.

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Potential Financial Conflicts of Interest: *Consultancies:* P. Lips (Merck & Co. Inc., Eli Lilly Inc., Wyeth, Novartis); *Honoraria:* P. Lips (Merck & Co. Inc., Eli Lilly Inc., Wyeth, Novartis, Servier); *Expert testimony:* P. Lips (Eli Lilly Inc.); *Grants received:* A.G. Uitterlinden (European Union), S.H. Ralston (European Union), P. Lips (Eli Lilly Inc., Merck & Co. Inc., Aventis, Wyeth); *Patents received:* A.G. Uitterlinden (Erasmus University Rotterdam), S.H. Ralston (University of Aberdeen); J.P.T.M. van Leeuwen (Erasmus University Rotterdam), H.A.P. Pols (Erasmus University Rotterdam).

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References

1. **Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al.** Prediction of bone density from vitamin D receptor alleles. Nature. 1994;367:284-7. Erratum in: Nature 1997 May 1;387:106. [PMID: 8161378]

2. **Uitterlinden AG, Pols HA, Burger H, Huang Q, Van Daele PL, Van Duijn CM, et al.** A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. J Bone Miner Res. 1996;11:1241-8. [PMID: 8864898]

3. **Cooper GS, Umbach DM.** Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Miner Res. 1996;11:1841-9. [PMID: 8970884]

4. **Thakkinstian A, D'Este C, Eisman J, Nguyen T, Attia J.** Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. J Bone Miner Res. 2004;19:419-28. [PMID: 15040830]

5. **Gong G, Stern HS, Cheng SC, Fong N, Mordeson J, Deng HW, et al.** The association of bone mineral density with vitamin D receptor gene polymorphisms. Osteoporos Int. 1999;9:55-64. [PMID: 10367030]

6. **Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, et al.** The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. J Bone Miner Res. 2001;16:1256-64. [PMID: 11450701]

7. **Fang Y, van Meurs JB, Bergink AP, Hofman A, van Duijn CM, van Leeuwen JP, et al.** Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. J Bone Miner Res. 2003;18:1632-41. [PMID: 12968672]

8. **Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D.** The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res. 1996;11:1850-5. [PMID: 8970885]

9. **Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, et al.** Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. JAMA. 2004;292:2105-14. [PMID: 15523071]

10. **Burger H, de Laet CE, van Daele PL, Weel AE, Witteman JC, Hofman A, et al.** Risk factors for increased bone loss in an elderly population: the Rotterdam Study. Am J Epidemiol. 1998;147:871-9. [PMID: 9583718]

11. **Torgerson DJ, Thomas RE, Campbell MK, Reid DM.** Randomized trial of osteoporosis screening. Use of hormone replacement therapy and quality-of-life results. Arch Intern Med. 1997;157:2121-5. [PMID: 9382669]

12. **Pluijm SM, van Essen HW, Bravenboer N, Uitterlinden AG, Smit JH, Pols HA, et al.** Collagen type I alpha1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women. Ann Rheum Dis. 2004;63:71-7. [PMID: 14672895]

13. **Ismail AA, O'Neill TW, Cooper C, Finn JD, Bhalla AK, Cannata JB, et al.** Mortality associated with vertebral deformity in men and women: results from the European Prospective Osteoporosis Study (EPOS). Osteoporos Int. 1998;8:291-7. [PMID: 9797915]

14. **Ralston SH, Galwey N, MacKay I, Albagha OM, Cardon L, Compston JE, et al.** Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. Hum Mol Genet. 2005;14:943-51. [PMID: 15746152]

15. **Kudlacek S, Schneider B, Peterlik M, Leb G, Klaushofer K, Weber K, et al.** Normative data of bone mineral density in an unselected adult Austrian population. Eur J Clin Invest. 2003;33:332-9. [PMID: 12662164]

16. **McCloskey EV, Spector TD, Eyres KS, Fern ED, O'Rourke N, Vasikaran S, et al.** The assessment of vertebral deformity: a method for use in population studies and clinical trials. Osteoporos Int. 1993;3:138-47. [PMID: 8481590]

17. **Reid DM, Mackay I, Wilkinson S, Miller C, Schuette DG, Compston J, et al.** Cross-calibration of dual-energy X-ray densitometers for a large, multi-center genetic study of osteoporosis. Osteoporos Int. 2006;17:125-32. [PMID: 16136277]

18. **Stephens M, Donnelly P.** A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003;73:1162-9. [PMID: 14574645]

19. **McCulloch CE, Searle SR.** Generalized, Linear, and Mixed Models. New York: Wiley; 2001.

20. **Pettiti D.** Meta-Analysis, Decision Analysis and Cost-Effectiveness Analysis. Oxford: Oxford Univ Pr; 1999.

21. **Higgins JP, Thompson SG, Deeks JJ, Altman DG.** Measuring inconsistency in meta-analyses. BMJ. 2003;327:557-60. [PMID: 12958120]

22. **DerSimonian R, Laird N.** Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177-88. [PMID: 3802833]

23. **Rousset F, Raymond M.** Testing heterozygote excess and deficiency. Genetics. 1995;140:1413-9. [PMID: 7498780]

24. **Fang Y, van Meurs JB, d'Alesio A, Jhamai M, Zhao H, Rivadeneira F, et al.** Promoter and 3'-untranslated-region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: the Rotterdam study. Am J Hum Genet. 2005;77:807-23. [PMID: 16252240]

25. **Rebbeck TR, Spitz M, Wu X.** Assessing the function of genetic variants in candidate gene association studies. Nat Rev Genet. 2004;5:589-97. [PMID: 15266341]

26. **Little J, Khoury MJ.** Mendelian randomisation: a new spin or real progress? Lancet. 2003;362:930-1. [PMID: 14511923]

27. **Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P, et al.** Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS study. PLoS Med. 2006;3:e90. [PMID: 16475872]

28. **Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG.** Genetic associations in large versus small studies: an empirical assessment. Lancet. 2003;361:567-71. [PMID: 12598142]

29. **Little J, Khoury MJ, Bradley L, Clyne M, Gwinn M, Lin B, et al.** The human genome project is complete. How do we develop a handle for the pump? [Editorial] Am J Epidemiol. 2003;157:667-73. [PMID: 12697570]

30. **Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG.** Replication validity of genetic association studies. Nat Genet. 2001;29:306-9. [PMID: 11600885]

31. **Ioannidis JP.** Genetic associations: false or true? Trends Mol Med. 2003;9:135-8. [PMID: 12727138]

32. **Ioannidis JP.** Why most published research findings are false. PLoS Med. 2005;2:e124. [PMID: 16060722]

33. **Pan Z, Trikalinos TA, Kavvoura FK, Lau J, Ioannidis JP.** Local literature bias in genetic epidemiology: an empirical evaluation of the Chinese literature. PLoS Med. 2005;2:e334. [PMID: 16285839]

34. **Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N.** Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst. 2004;96:434-42. [PMID: 15026468]

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Collection and assembly of data: A.G. Uitterlinden, S.H. Ralston, M.L. Brandi, A.H. Carey, D. Grinberg, B.L. Langdahl, B. Obermayer-Pietsch, J. Reeve, D.M. Reid, A. Bassiti, M. Bustamante, L.B. Husted, A. Diez-Perez, A. Fahrleitner-Pammer, Y. Fang, E. Karczmarewicz, M. Kruk, J.B.J. van Meurs, J. Mangion, F.E.A. McGuigan, H.A.P. Pols, W. Renner, F. Rivadeneira, S. Scollen.

Appendix Table. Study Sample Characteristics and Genotype and Haplotype Distribution*

Characteristic	Aarhus		Aberdeen	Amsterdam		Barcelona	EPOS	
Design	Case–control		Cohort	Cohort		Cross-sectional	Cohort	
Sex, *n*	F: 588	M: 157	F: 3886	F: 785	M: 728	F: 757	F: 2513	M: 1733
Mean age (SD), *y*	61.4 (13.2)	53.5 (15.8)	48.5 (2.4)	76.0 (6.7)	75.9 (6.6)	54.8 (8.6)	64.9 (10.8)	64.4 (12.2)
Mean height (SD), *cm*	161.6 (6.6)	176.4 (7.3)	161.2 (5.9)	160.0 (6.4)	173.0 (6.8)	156.3 (6.3)	158.3 (6.6)	171.1 (7.2)
Mean weight (SD), *kg*	64.2 (10.9)	78.4 (12.2)	66.2 (12.2)	70.8 (13.0)	77.9 (11.9)	64.7 (10.1)	67.9 (11.8)	79.3 (11.5)
Mean BMI (SD), kg/m^2	24.6 (4.1)	25.3 (3.7)	25.5 (4.5)	27.7 (4.9)	26.0 (3.4)	26.5 (3.9)	27.1 (4.7)	27.1 (3.6)
Postmenopausal, *n (%)*	486 (82.7)	Not pertinent	1779 (46.1)	781 (100)	Not pertinent	748 (100)	2082 (88.4)	Not pertinent
HRT, %	0 (0.0)	Not pertinent	2266 (58.6)	101 (13.0)	Not pertinent	0 (0.0)	306 (15.6)	Not pertinent
Activity or ability†								
Index range	No data	No data	0.76–3.26	0–624	0–625	No data	7–24	7–24
Median	No data	No data	1.77	156	102	No data	21	23
Mean BMD								
Method‡	Hologic (Cal)	Hologic (Cal)	Norland	Hologic	Hologic	Hologic	Various (ESP)	Various (ESP)
Lumbar spine (SD), *g/cm*^{2}	0.827 (0.179)	0.829 (0.178)	1.052 (0.161)	0.915 (0.167)	1.036 (0.189)	0.861 (0.154)	0.928 (0.190)	1.032 (0.185)
Participants with lumbar spine data, *n*	567	155	3882	274	260	753	861	546
Femoral neck (SD), *g/cm*^{2}	0.671 (0.129)	0.683 (0.116)	0.881 (0.125)	0.662 (0.107)	0.742 (0.136)	0.687 (0.111)	0.727 (0.137)	0.850 (0.150)
Participants with femoral neck data, *n*	561	154	3881	263	259	628	2008	1497
Any fracture, *n (%)*	247 (42.0)	56 (35.7)	784 (20.2)	391 (63.2)	327 (59.0)	113 (21.0)	815 (32.6)	407 (23.6)
Incident fracture, *n (%)*			344 (9.0)	150 (19.2)	87 (12.0)		323 (16.7)	73 (5.7)
No- or low-trauma fracture, *n (%)*	247 (42.0)	56 (35.7)	No data	190 (38.0)	160 (33.8)	113 (21.0)	815 (32.6)	407 (23.6)
Vertebral fracture, *n (%)*	247 (42.0)	56 (35.7)	16 (0.4)	136 (50.9)	128 (49.2)	52 (9.6)	228 (9.1)	123 (7.1)
Incident fracture, *n (%)*			6 (0.2)‡	21 (6.6)	16 (5.4)		67 (3.7)	21 (1.6)
Genotype, *n (%)*								
Cdx2								
GG	380 (66.0)	114 (73.1)	1999 (64.0)	296 (63.1)	316 (69.8)	365 (57.4)	1612 (64.5)	1079 (62.8)
GA	170 (29.5)	39 (25.0)	985 (31.6)	147 (31.3)	122 (26.9)	228 (35.8)	787 (31.5)	570 (33.2)
AA	26 (4.5)	3 (1.9)	137 (4.4)	26 (5.5)	15 (3.3)	43 (6.8)	99 (4.0)	68 (4.0)
FokI								
CC	216 (37.0)	6 (38.2)	1186 (38.5)	185 (39.6)	174 (38.5)	293 (43.0)	969 (38.9)	614 (35.9)
CT	271 (46.5)	70 (44.6)	1423 (46.1)	206 (44.1)	218 (48.2)	305 (44.7)	1157 (46.3)	832 (48.6)
TT	96 (16.5)	27 (17.2)	475 (15.4)	76 (16.3)	60 (13.3)	84 (12.3)	368 (14.8)	265 (15.5)
Haplotype alleles, *n (%)*§								
BsmI–ApaI–TaqI								
1. *G-G-T*	477 (41.6)	133 (43.2)	2577 (41.8)	424 (47.3)	396 (45.5)	501 (42.9)	2325 (47.6)	1666 (49.3)
2. *A-T-C*	498 (43.4)	124 (40.3)	2310 (37.4)	375 (41.9)	361 (41.5)	430 (36.8)	1888 (38.7)	1288 (38.2)
3. *G-T-T*	98 (8.5)	36 (11.7)	805 (13.0)	88 (9.8)	100 (11.5)	124 (10.6)	588 (12.0)	386 (11.4)
4. *A-T-T*	36 (3.1)	12 (3.9)	149 (2.4)	8 (0.9)	10 (1.1)	39 (3.3)	61 (1.2)	27 (0.8)
5. *G-G-C*	6 (0.5)	1 (0.3)	155 (2.5)	1 (0.1)	2 (0.2)	30 (2.6)	14 (0.3)	6 (0.2)
6. *A-G-T*	17 (1.5)	2 (0.6)	164 (2.7)	0	1 (0.1)	34 (2.9)	6 (0.1)	3 (0.1)
7. *G-G-C*	14 (1.2)	0	3 (0.0)	0	0	6 (0.5)	1 (0.02)	0
8. *A-G-C*	2 (0.2)	0	7 (0.1)	0	0	4 (0.3)	1 (0.02)	0
Probability <0.95, *n (%)*‖	5 (1)	1 (0.8)	44 (1.7)	0	0	16 (3.2)	0	0

* Reported percentages are estimated on the basis of participants with available data for the respective characteristic. Oxagen represents the Familial Osteoporosis Study (FAMOS). European Polish Osteoporosis Study data are combined with EPOS. BMD = bone mineral density; BMI = body mass index; Cal = calibration of a few Norland values to Hologic equivalents; EPOS = European Prospective Osteoporosis Study; ESP = European Spine Phantom calibration; F = female; HRT = hormone replacement therapy (at any time up to the time of BMD measurement); M = male.

† Data for physical activity and ability were measured in different scales capturing various activities and abilities across cohorts.

‡ Hologic bone densitometers are manufactured by Hologic, Bedford, Massachusetts; Norland densitometers are manufactured by Cooper Surgical, Trumbull, Connecticut; and Lunar densitometers are manufactured by GE Medical Systems, Madison, Wisconsin.

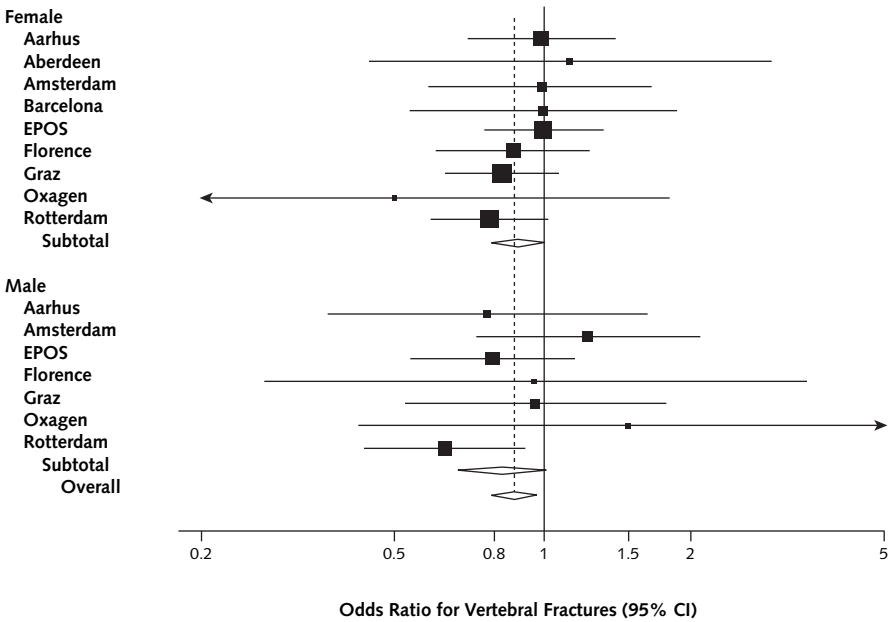
§ Haplotype alleles are numbered 1 to 8 by decreasing frequency and are given as a string of nucleotide alleles at the 3 adjacent variant sites for *Bsm*I, *Apa*I, and *Taq*I, respectively.

‖ Data in which haplotypes were inferred with credibility less than 95%.

Appendix Table—Continued

Florence		Graz		Oxagen		Rotterdam	
Cross-sectional		Cross-sectional		Cross-sectional		Cohort	
F: 1724	M: 631	F: 1464	M: 415	F: 341	M: 221	F: 4878	M: 3105
62.1 (12.5)	62.1 (16.3)	70.8 (20.5)	67.4 (17.0)	46.8 (14.7)	48.6 (14.9)	70.1 (9.3)	68.4 (8.2)
158.0 (7.1)	170.1 (8.4)	157.5 (8.6)	172.4 (9.1)	163.2 (6.9)	177.6 (7.2)	161.1 (6.7)	174.6 (6.8)
63.9 (10.7)	76.7 (12.5)	61.9 (11.6)	77.6 (13.9)	66.0 (14.3)	80.8 (13.3)	69.3 (11.4)	78.2 (10.8)
25.6 (4.3)	26.5 (3.6)	25.0 (4.6)	26.0 (3.8)	24.7 (4.5)	25.6 (3.9)	26.7 (4.1)	25.6 (3.0)
1587 (92.2)	Not pertinent	1173 (80.1)	Not pertinent	158 (46.3)	Not pertinent	4865 (99.7)	Not pertinent
107 (6.2)	Not pertinent	71 (4.8)	Not pertinent	79 (23.2)	Not pertinent	692 (15.0)	Not pertinent
No data	No data	0–4	0–4	3–14	3–14	0–3	0–3
No data	No data	1	1	7.00	8.33	2.75	2.88
Hologic	Hologic	Hologic	Hologic	Various (ESP)	Various (ESP)	Lunar	Lunar
0.868 (0.176)	1.002 (0.167)	0.940 (0.152)	1.013 (0.144)	1.000 (0.205)	1.088 (0.247)	1.036 (0.180)	1.165 (0.197)
1677	622	512	236	335	215	3381	2463
0.681 (0.143)	0.857 (0.175)	0.751 (0.120)	0.855 (0.120)	0.775 (0.150)	0.846 (0.171)	0.811 (0.131)	0.876 (0.133)
784	118	512	241	336	217	3239	2435
270 (15.9)	76 (12.0)	577 (39.4)	172 (41.4)	131 (38.9)	104 (47.7)	1197 (24.5)	400 (12.9)
						1113 (22.8)	317 (10.2)
270 (15.9)	76 (12.0)	No data	No data	79 (23.4)	47 (21.5)	1014 (20.8)	269 (8.7)
129 (7.6)	10 (1.6)	401 (27.4)	60 (14.5)	14 (4.2)	11 (5.0)	316 (15.5)	161 (10.6)
						213 (4.4)	68 (2.2)
943 (55.3)	365 (59.0)	956 (71.7)	258 (67.2)	218 (65.5)	125 (59.0)	2486 (65.5)	1727 (66.6)
660 (38.7)	231 (37.3)	342 (25.6)	120 (31.3)	101 (30.3)	77 (36.3)	1165 (30.7)	776 (29.9)
102 (6.0)	23 (3.7)	36 (2.7)	6 (1.6)	14 (4.2)	10 (4.7)	145 (3.8)	89 (3.4)
723 (42.5)	243 (39.1)	535 (36.6)	150 (40.9)	143 (42.9)	78 (36.8)	1527 (40.1)	1037 (39.8)
783 (46.0)	293 (47.2)	721 (49.3)	179 (48.8)	143 (42.9)	100 (47.2)	1801 (47.2)	1227 (47.1)
197 (11.6)	85 (13.7)	207 (14.1)	38 (10.4)	47 (14.1)	34 (16.0)	484 (12.7)	340 (13.1)
1406 (42.3)	486 (40.6)	1113 (48.9)	304 (49.5)	1423 (45.1)	839 (45.3)	3472 (47.3)	2387 (47.4)
1359 (40.9)	537 (44.9)	766 (33.7)	204 (33.2)	1254 (39.7)	733 (39.5)	2965 (40.4)	2048 (40.7)
487 (14.7)	145 (12.1)	286 (12.6)	91 (14.8)	294 (9.3)	169 (9.1)	832 (11.3)	559 (11.1)
58 (1.7)	22 (1.8)	42 (1.8)	6 (1.0)	78 (2.5)	39 (2.1)	42 (0.6)	28 (0.6)
13 (0.4)	6 (0.5)	3 (0.1)	1 (0.2)	58 (1.8)	39 (2.1)	18 (0.2)	9 (0.2)
0	0	59 (2.6)	7 (1.1)	32 (1.0)	12 (0.6)	3 (0.0)	4 (0.1)
1 (0.03)	0	5 (0.2)	1 (0.2)	19 (0.6)	23 (1.2)	1 (0.01)	1 (0.02)
0	0	0	0	0	0	1 (0.01)	0
0	0	18 (1.6)	4 (1.3)	52 (3.7)	28 (3.3)	0	0

Appendix Figure. **Odds ratio for vertebral fractures for the Cdx2 polymorphism according to the dominant genetic model.**



Point estimates and 95% CIs are shown per study and for summary estimates. EPOS = European Prospective Osteoporosis Study.