Genetic Effects of Estrogen Receptor α and Collagen IA1 Genes on the Relationships of Parathyroid Hormone and 25 Hydroxyvitamin D With Bone Mineral Density in Caucasian Women

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There is a growing body of evidence that estrogen receptor α (*ER*α) and collagen IA1 (*COLIA1*) genes may affect bone mineral density (BMD) levels in postmenopausal women. In a recent study we found that the Px haplotype of the *ER*α gene (resulting from combined *Pvu*II and *Xba*I restriction fragment-length polymorphisms [RFLPs] in intron 1) was associated with low radiographic phalangeal hand BMD in elderly women (62.7 ± 6.5 years of age), of European origin. The combination of the Px haplotype and "s" allele of the *COLIA1* gene (*Msc*I RFLP in Sp1 locus) decreased BMD in these women. The major aim of the present study was to investigate whether the genetic effects of these genotypes on cancellous and cortical hand BMD, in the same elderly women (N = 122), are possibly mediated through circulating levels of parathyroid hormone (PTH) and/or 25 hydroxyvitamin D [25(OH)D], and may be related to biochemical markers of bone turnover (propeptide of type I procollagen [PICP] and osteocalcin). Multiple regression analyses of age-adjusted cancellous BMD revealed that *ER*α polymorphism and circulating levels of PTH were independent predictors of about 12.9% of its variation. Some 17.9% of cortical BMD variations were attributable to the combined effects of *ER*α polymorphism and plasma concentrations of 25(OH)D, estradiol, and PTH. The significant inverse association between PTH and BMD of both types was further confirmed by association analysis according to categorical subgroups of BMD values, as well by haplotype status. The mean difference in PTH concentrations between subjects carrying the Px haplotype (higher mean) and those lacking it (lower mean) reached 0.59 SD (*P* = .01). The difference in PTH levels further increased when explored in the 4 subgroups formed by combinations of polymorphic *ER*α and *COLIA1* genotypes. Mean PTH of subjects carrying both the Px haplotype and "s" allele was higher by 1.52 SD (*P* = .001) than in subjects lacking both the Px haplotype and "s" allele. Those carrying both Px haplotype and "s" allele were also characterized by highest mean value of PICP and lowest means of 25(OH)D and BMD (both tissue types). We conclude that in the studied elderly women, the Px haplotype may be involved in causing the phenotypic expression of higher circulating levels of PTH and higher bone turnover, which, in turn, may lead to bone loss.

BONE mineral density (BMD) is a quantitative trait determined by the interaction of genetic, metabolic, and environmental factors, and its diminution is deemed a major risk factor for fractures. To date, several candidate genes have received great attention, among them the collagen IA1 (*COLIA1*) and estrogen receptor α (*ER*α) genes.[1-8] The *COLIA1* gene encodes the most abundant bone matrix protein, collagen type Iα1. Estrogen receptor genes mediate in women the effects of estrogen, probably the major systemic hormone for maintaining normal bone turnover,[9] and its deficiency leads to bone mass loss.[10] While there are some evidence that the *ER*α gene may be involved in regulation of BMD in men,[11,12] the main evidence for its regulatory role of BMD in women derives from studies performed in postmenopausal women of different ethnic origins.[5,7,8,13]

We recently conducted haplotype-based association analysis between combined *Pvu*II and *Xba*I restriction fragment-length polymorphisms (RFLPs) in intron 1 of *ER*α gene and phalangeal hand BMD, on pedigree data collected from a population of European origin, residing in Russia.[13] We found that the Px haplotype of the *ER*α was associated with low cancellous and cortical BMD in a maternal subgroup, actually comprised of elderly women (62.7 ± 6.5 years of age). The transmission disequilibrium test (TDT) revealed evidence suggestive of a linkage disequilibrium between the Px haplotype of the *ER*α gene and the BMD putative gene in female (but not in male) offspring. Additionally in our elderly women sample, the "s" allele of *COLIA1* gene substantially enhanced the influence of the Px haplotype of the *ER*α gene by further reducing BMD in subjects carrying the combined genotypes (Px haplotype and "s" allele).

It has been shown that increased/decreased levels of calciotropic hormones (parathyroid hormone [PTH] and 25 hydroxyvitamin D [25(OH)D]), and biochemical indices of bone formation like the carboxyterminal propeptide of type I procollagen (PICP), or osteocalcin, may be associated with increased risk for osteoporosis fracture in postmenopausal women, independently of each other or of BMD.[14-16] The present study was therefore undertaken to evaluate whether the association of the Px haplotype of the intronic *ER*α gene and allele "s" of Sp1 locus in the *COLIA1* gene with phalangeal hand BMD could be related to calciotropic hormones and/or biochemical indices of bone formation. Specifically we addressed the following 4 questions: (1) Is there an association between high or low levels of each of the above biochemical indices and low BMD? (2) Is there an association between high or low levels of each of the above biochemical indices and either or both the Px haplotype of the *ER*α gene and the "s" allele of the *COLIA1* gene? (3) Is there a specific genotype whose carriers are characterized by a lowest mean BMD and highest (or lowest) circulating levels of

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the biochemical indices? (4) What is the quantitative contribution of the Px haplotype and other studied variables to the variance of BMD in elderly women?

MATERIALS AND METHODS

Subjects and BMD Measurements

The Chuvashaian population chosen for our study is ethnically a Caucasian population living in numerous small villages in the forested or hilly portions of the Volga riverside in Russia. Their ancestors were most likely Bulgars from the Volga and Kama riverside who intermarried with the local Finno-Ugor tribes. The individuals assessed in the present research were part of a pedigree sample that resided in the area for at least 3 generations. Sharing a similar environment, as well as similar socioeconomic conditions, and also a minimal genetic flow, is what characterizes this rural population, which has been described by us in greater detail elsewhere.[17,18] The population does not have access to modern medical services, nor to the use of hormone replacement therapy or calcium supplements. Our study did not include individuals with known bone diseases or with risk factors for increased BMD loss, such as steroid hormone therapy, diabetes, or hyperparathyroidism. Our research team randomly selected families for study after direct contact with all the households in the small villages. The subjects recruited for study were all members of nuclear families who agreed to participate and signed on informed consent. The project was approved by the Tel-Aviv University ethics committee and encompassed 122 elderly women from the total pedigree-based sample (463 individuals). The mean age (± SD) of these women, representing the maternal moiety of the original pedigrees, was 62.7 years (±6.5) and mean of years since menopause (± SD) was 14.6 years (±7.4). Plain radiographs of both hands were obtained from each individual with an x-ray source. BMD of cancellous and cortical bone separately, at the distal and middle phalanges of the third finger on both hands, was evaluated by digital microdensitometer, using a standard methodology.[18-20] We used average BMD measure of the third finger bones of both hands, and calculated separately the *Z* score values for cancellous bone and cortical bone, as previously described.[17,18] We should note that the *Z*-transformation was based on the data of the total pedigrees sample (>700 individuals), and took into account size and structure of the studied families.

Determination of ERα and COLIA1 Genotypes

Genomic DNA, extracted from peripheral leukocytes by either standard phenol-chloroform procedure or via a Nucleon kit (Amersham Life Science, London, UK), was used for specific polymerase chain reaction (PCR) amplification. The PCR product of the *ERα* gene (~1.3-kb fragment), containing a part of intron 1 and exon 2, was amplified according to the Kobayashi et al protocol.[5] To analyze the *Pvu*II and *Xba*I RFLPs in this fragment, we used direct haplotyping procedure. Haplotypes of *ERα* gene represented the combination of both polymorphic sites (P/p and X/x) on each of the sixth pair chromosomes in each individual. Direct haplotype analysis was enabled by simultaneously digesting the PCR product with the 2 restriction enzymes, *Pvu*II and *Xba*I, as per our recent report.[12] Absence or presence of the sites for the restriction enzymes *Pvu*II and *Xba*I was recorded as P or p and X or x respectively. Genotypes by haplotypes were determined for each subject as PXPX, pxpx, etc. The PCR product of the *COLIA1* gene (~260-bp fragment) was amplified by using mismatched primer as previously described,[1] taking into account that the fragment contains an introduced restriction site for *Msc*I enzyme, at the Sp1 binding site in the first intron of the gene. Absence or presence of the *Msc*I restriction site was recorded as S or s, respectively.

In the sample of unrelated parents, both "mothers" and "fathers," the genotype frequency distribution at both loci, ie, *Pvu*II and *Xba*I RFLPs in the *ERα* gene and *Msc*I RFLP in the *COLIA1* gene, was in conformity with the Hardy-Weinberg equilibrium.[13]

Determination of Plasma Levels of Bone Turnover Markers, Calciotropic Hormones, and Steroid Sex Hormones

Plasma was separated from whole blood samples (collected in the morning after a 12-hour fast, during the autumn months) and stored at −80°C until assay. Plasma-intact osteocalcin was measured by immunoradiometric assay using ELSA-OSTEO kit (CIS Bio International, ORIS Group, France). Carboxyterminal PICP was measured by radioimmunoassay, using the ^{125}I- RIA kit (DiaSorin, Stillwater, MN). Intact (I-)PTH was measured by immunoradiometric assay, using N-tact PTH SP kit (Incstar, Stillwater, MN). 25(OH)D was assayed by radioimmunoassay with the ^{125}I-RIA kit (DiaSorin). In this assay there is virtually no cross reactivity with vitamin D_3, or its metabolite $1,25(OH)_2D_3$. Total testosterone and estradiol levels were determined by radioimmunoassay using TESTO-CT2 and ESTR-US-CT kits (CIS Bio International). The intra-assay and interassay coefficients of variation (CVs) for the above kits varied from 3.2% to 12.5% and from 4.9% to 17.6%, respectively. The kit detection limit for estradiol had been assessed as 5 pmol/L. All assays were performed in duplicate and the results were reported in detail.[21]

Statistical Analysis

Plasma levels of each of the biochemical indices were age adjusted and standardized within the "mothers" subgroup of the pedigree sample in order to achieve a mean equaling 0 and a SD of 1.0 (*Z* values). To test for any association between high or low levels of BMD and each of the potential predictive variables, we first divided the BMD *Z* scores (ranging under normal distribution from −2.5 to 2.3 *Z* scores) into 3 subgroups as follows: A = $Z < -1$, B = $-1 \le Z \le 1$, and C = $Z > 1$, for each cancellous and cortical bone tissue. The mean values of all the hormones and biochemical markers in these 3 BMD subgroups were then compared by 1-way analysis of variance (ANOVA). Student's *t* test was used to test the hypothesis that a candidate gene polymorphism exerts an influence on calciotropic hormones or bone turnover markers. Additionally, when combined di-loci genotypes were used for similar comparisons, ANOVA was implemented. Forward stepwise multiple regression analysis was performed to evaluate the independent contribution of the potential predictor variables for *Z* scores (as continuous variable) of each of the 2 BMD types. The calciotropic hormones, sex steroid hormones and bone turnover markers, were introduced as quantitative variables (age-adjusted standardized residuals). Genotypes represented by haplotypes as categorical variables were entered as "dummy" variables by coding them numerically: "1" for subjects carrying 1 or 2 copies of the Px haplotype (genotypes PxPx, PxPX, Pxpx) and "2" for subjects lacking this haplotype (genotypes PXPX, PXpx, pxpx). For the sake of convenience some additional explanations will be given in the relevant text of the Results section. All the above analyses were performed using the STATISTICA package for Windows (version 5.0, StatSoft, 2000).

RESULTS

The plasma levels of PTH, 25(OH)D, PICP, osteocalcin, estradiol and testosterone are shown in Table 1. Mean age-adjusted PTH levels differed significantly between the subgroups of categorical BMD *Z* scores of both types of bone tissue, with the highest values observed in the subgroup of $Z < -1$, and the lowest in subgroup $Z > 1$ (Fig 1). Similar examination of all the other biochemical variables vis-a-vis cancellous BMD evinced that between subgroups, only the testosterone concentrations showed marginally significant differences

Table 1. Descriptive Statistics for Studied Sample

Variable	N	Mean	Minimum	Maximum	SD
Age (yr)	122	62.7	45	79	6.5
Cancellous BMD (*Z* scores)	122	0	−2.50	2.34	1.0
Cortical BMD (*Z* scores)	122	0	−2.51	2.35	1.0
PTH (pg/mL)	117	37.01	7.50	78.25	15.80
25 (OH)D (ng/mL)	122	11.67	4.90	32.65	6.86
PICP (ng/mL)	120	128.81	54.00	218.90	34.37
Osteocalcin (ng/mL)	119	18.48	4.10	37.05	7.54
Estradiol (pg/mL)	117	35.89	<1.40	189.10	36.52
Testosterone (ng/mL)	119	1.07	0.10	3.40	0.52

($P = .059$), whereas the other indices were unremarkable. The same examination for cortical bone showed that 25(OH)D levels correlated negatively with BMD, while circulating testosterone decreased in parallel with cortical BMD decrease (Fig 2).

Figure 3 clearly demonstrates negative correlation between BMD (both types) and PTH, in line with the Px haplotype status. In other words, subjects who carried 1 or 2 copies of the Px haplotype (combined group of PxPx PxPX, Pxpx genotypes) had significantly higher levels of PTH and lower values of BMD (as a continuous variable), than subjects lacking this haplotype (PXPX, PXpx, pxpx genotypes). The difference attained 0.59 *Z* score ($P = .010$) for PTH, and 0.78 *Z* score ($P = .003$), and 0.60 *Z* score ($P = .025$) for cancellous and cortical bone, respectively. No significant differences were noted for 25(OH)D, estradiol, and testosterone. Levels of the bone formation markers PICP and osteocalcin displayed the same trend as PTH (as expected), but the differences here did not reach statistical significance. We also assessed the predictive power of the Px haplotype and each of the age-adjusted levels of hormones [PTH, 25(OH)D, estradiol, and testosterone] and biochemical markers (PICP or osteocalcin) on age-adjusted BMD, using the multiple regression analysis (Table 2). Table 2 provides a simultaneous *F* test of the hypothesis that all the regression coefficients are equal to zero, and individual *t* tests for each coefficient separately. As can be seen in Table 2, the overall multivariate *F* ratio was highly significant ($P < .001$) in both the undertaken analyses. However, different arrays of predictor variables were retained for cancellous and cortical BMD. The regression model explained 12.9% of the total observed variance of cancellous BMD (Table 2). PTH and the carrying of Px haplotype were the most significant independent predictors for low BMD values, with some 7.6% ($P = .004$) and 4.1% ($P = .028$) of the BMD variance attributable to PTH levels and genotype. The contributions of testosterone and osteocalcin to the predictive power of the model were only marginally significant ($.05 < P < .11$). Multiple regression analysis of cortical BMD revealed that about 18% of the total variance was explained by the selected set of variables. The results of the analysis suggested that 25(OH)D and possession of the Px haplotype exerted a most substantial effect (7.5% and 6.2%, respectively). The rest was explained by the estradiol and PTH effects. Osteocalcin made no significant ($P = .228$) contribution to the BMD variation, yet its exclusion from the analysis significantly diminished the overall likelihood of the model fit.

Next we attempted to assess possible associations between each of the hormones and biochemical markers and the com-

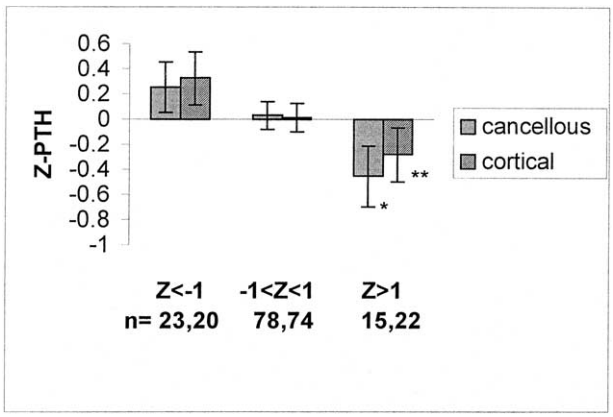

Fig 1. ***Z*** **means (SEM) of PTH, according to 3 categorized** ***Z*** **scores of cancellous or cortical bone; comparisons are between group** $Z < -1$ ***v*** $Z > 1$ **(Duncan test): *P = .017; **P = .031.**

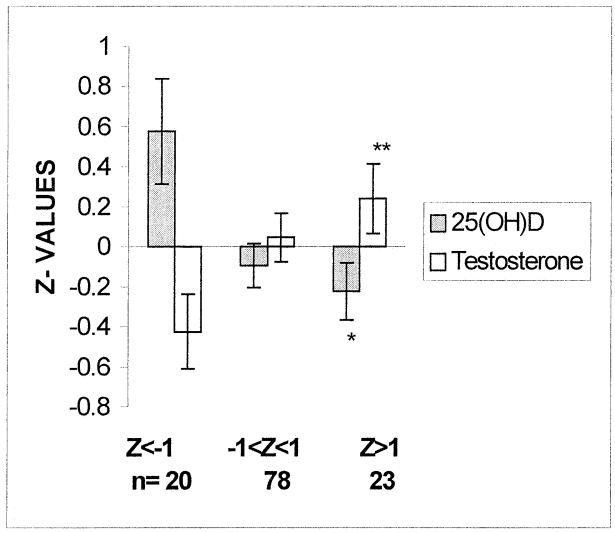

Fig 2. ***Z*** **means (SEM) of 25(OH)D, testosterone according to 3 categorized** ***Z*** **scores of cortical bone; comparisons are between group** $Z < -1$ ***v*** $Z > 1$ **(Duncan test): *P = .003; **P = .017.**

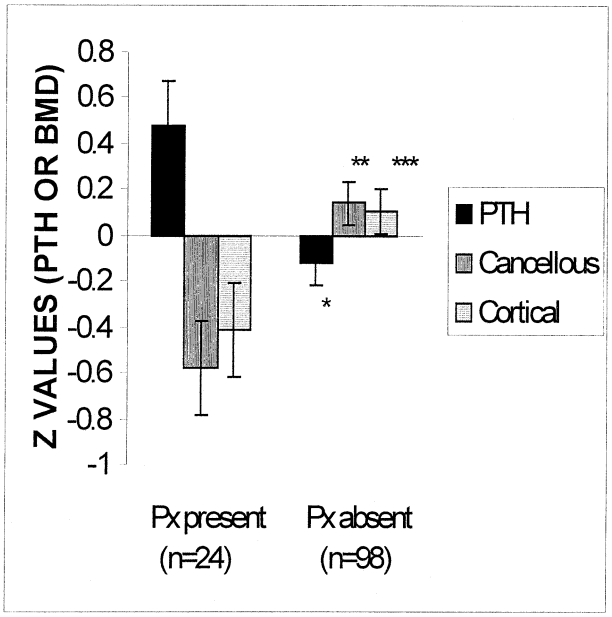

Fig 3. *Z* means (SEM) of PTH, and cancellous and cortical BMD, according to Px haplotype status; *t* test: **P* = .010; ***P* = .003; ****P* = .025.

bination of genotypes at both polymorphic *ERα* and *COLIA1* genes. To this end, analyses of variance for the alternative combinations of these di-loci genotypes were conducted. We designated as "1" the genotypes of *ERα* carrying 1 or 2 copies of the Px haplotype, and as "2" the genotypes lacking the Px haplotype. The genotypes of *COLIA1* carrying one or two copies of "s" allele (ie, Ss and ss) were defined as "A" and the SS genotype as "B." Altogether the combinations formed 4 genotypic groups as follows: "1A," "2A," "1B," "2B." Figure 4 shows that women of group 1A had the lowest BMD *Z* scores for both cancellous and cortical bone, the lowest mean 25(OH)D, and the highest means for PTH, PICP, and osteocalcin. In this group, PTH was inversely correlated with both types of BMD and with 25(OH)D. Women with the alternative combination of genotypes (group 2B), had BMD (both cancellous and cortical bone), 25(OH)D, PTH, PICP, and osteocalcin values close to $Z = 0$, which actually is the overall sample mean of all the 4 subgroups of women. The differences between these 2 groups reached statistical significance for cancellous BMD and PTH ($P < .03$ and $P < .001$, respectively), but not for the other variables, namely, cortical BMD ($P = .087$), 25(OH)D ($P = .077$), PICP ($P = .119$), or osteocalcin ($P = .548$). The sex hormones (data not shown) had the lowest values within group 1A, and values around the median (mean $Z = 0$) within group 2B. The later differences, however, were not significant. It can also be seen in Fig 4 that women lacking Px haplotype and carrying "s" allele (group 2A) differed significantly insofar as the values of their PTH, 25(OH)D, PICP and cancellous BMD from women carrying both Px haplotype and "s" allele (group 1A). The difference for osteocalcin did not attain significance ($P = .156$). In regard to the mentioned variables, we observed that the mean level of each in group1A was opposite to the mean in group 2A (higher or lower than the median value). This is best demonstrated for PICP where the difference was 1.2 *Z* ($P = .012$) between the 2 groups.

DISCUSSION

In an earlier phase of our ongoing pedigree-based study of the radiographic phalanges BMD in the Chuvasha population we observed a strong influence of the putative genetic factors on the interindividual variation of the BMD.[18] In subsquent research on this population sample we found that the Px haplotype of the *ERα* gene was associated with low phalanges BMD in elderly women, and that the combination of the Px

Table 2. Multiple Linear Regression Analysis of BMD for Cancellous and Cortical Bone in the Group of Elderly Women

Predictor	β	SE β	R^2	Δ R^2	*P* Value
Cancellous bone					
Intercept					.037
PTH	−0.202	0.093	0.076	0.076	.004
Px haplotype*	0.200	0.093	0.117	0.041	.028
Testosterone	0.174	0.091	0.138	0.022	.105
Osteocalcine	−0.152	0.091	0.161	0.022	.097
	Final adjusted $R^2 = 0.129$ $F(4,105) = 4.005$ $P < .00095$				
Cortical bone					
Intercept					.019
$25(OH)D_3$	−0.360	0.091	0.075	0.075	.004
Px haplotype*	0.224	0.090	0.137	0.062	.007
Estradiol	−0.192	0.089	0.177	0.040	.027
PTH	−0.180	0.090	0.206	0.029	.052
Osteocalcin	−0.109	0.090	0.217	0.011	.228
	Final adjusted $R^2 = 0.179$ $F(5,103) = 5.715$ $P < .00011$				

NOTE. BMD *Z* scores were modeled as a continuous variable. Age-adjusted plasma concentrations of PTH, $25(OH)D_3$, estradiol, testosterone, and osteocalcin were used as independent variables in the regression model. β and SEβ is regression coefficient and its standard error; R^2, multiple R^2; ΔR^3, change of multiple R^2 at each step of regression.

*The Px haplotype was grouped according to its presence: subjects with 1 or 2 copies of the haplotype were coded as group "1"; subjects who are lacking the haplotype were coded as group "2".

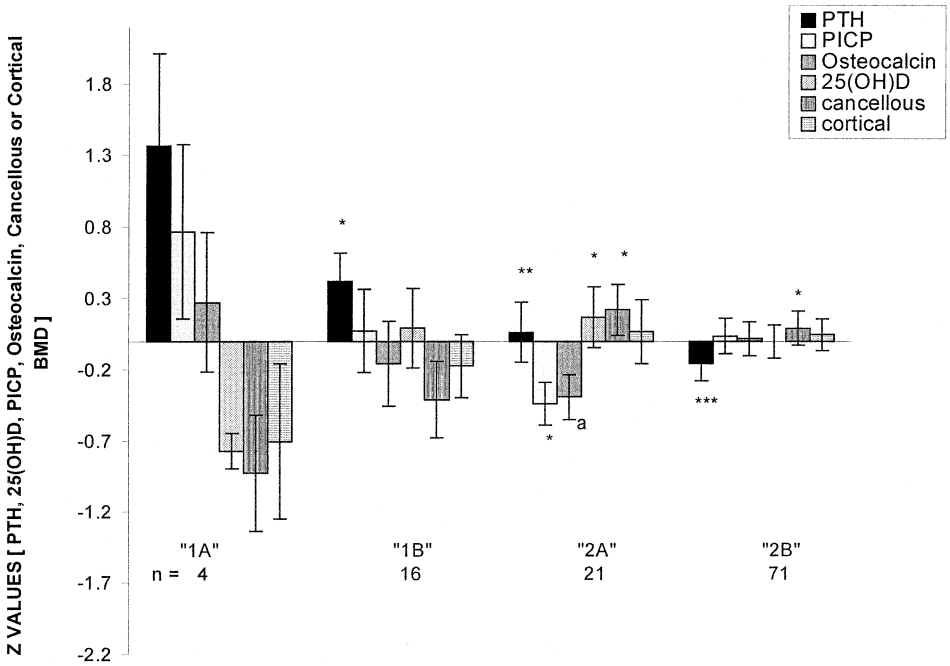

Fig 4. ***Z*** **means (SEM) of PTH, PICP, osteocalcin, 25(OH)D, and cancellous and cortical BMD, according to polymorphisms combinations of *ERα* gene (Px haplotype present or absent) and *COLIA1* gene (Ss and ss, or SS genotypes); subjects are grouped as follow: "1A" = carrying Px haplotype and carrying "s" allele; "1B" = carrying Px haplotype and not carrying "s" allele; "2A" = not carrying Px haplotype and carrying "s" allele; "2B" = not carrying Px haplotype and not carrying "s" allele; Duncan test (comparisons *v* group "1A"): *$P < .05$; **$P < .01$; ***$P < .001$; [a]not significant.**

haplotype and "s" allele of *COLIA1* gene further decreased the BMD.[13] In the present study, we have added biochemical predictors to the model, and used multiple regression analysis of BMD (dependent variable) adjusted for age, body height, and weight. As gleaned from Table 2, the model explained 12.9% and 17.9% of the remaining variance in the 2 types of BMD; furthermore, PTH and Px haplotype proved to be independent predictors of both types of BMD variation, while 25(OH)D and estradiol contributed additionally only to cortical bone variation.

Hormones and genes that are involved in bone metabolism constitute part of a very complex matrix of metabolic pathways and consequently can influence BMD in more than 1 pathway. As the hormones PTH, 25(OH)D, and estradiol, as well as the Px haplotype of the *ERα* gene, were all found to be independent predictors of BMD in our elderly women sample, we deemed it worthwhile to further investigate their interrelationships. Possession of the Px haplotype was associated both with higher PTH levels and lower BMD for the 2 bone tissue types. Moreover, this tendency became even better expressed when polymorphism at the *COLIA1* gene was taken into account, for then the highest mean value of PTH and the lowest mean BMD were observed in subjects carrying both Px haplotype and "s" allele (Fig 4). These findings suggest the possible interaction between *ERα* and *COLIA1* genes on the one hand, and on the other—that the effects of the *ERα* gene on BMD can be partially mediated by the PTH hormone [as well as by 25(OH)D]. Multiple regression analysis showed (Table 2) that both the *ERα* haplotype and PTH exerted an independent effect on BMD variation, assumedly through other actions on extraskeletal calcium homeostasis, as previously demonstrated.[22] This assumption is supported by the findings of Khosla et al[23] that serum estradiol is a weak but statistically significant predictor of PTH in postmenopausal women and by Heshmati et al,[24] who showed that Letrozole treatment in late postmenopausal women reduced serum estrone and estradiol to near undetectable levels, and decreased serum PTH by 22%. Since we could not detect correlation between circulating levels of estradiol and PTH, we tend to assume that the gamut of low levels of estradiol presented in our elderly women is exerting at least some of its actions through the transcription factor ERα, depending on the allele of *ERα* gene encoding it. While in subjects not carrying the Px haplotype, estrogen exerts its influence by at least maintaining BMD, subjects that do carry this haplotype, have an augmented response of PTH to estrogen (probably through the already demonstrated presence of estrogen receptors in the parathyroid glands[25]), which results in lower BMD. This explanation is receiving credence from emerging data that indicate that allelic variants in the *ERα* gene may modulate estrogen's effects, especially in regard to BMD[26,27] and lipid metabolism.[28-30]

Elevated levels of serum PTH have been considered to be associated with high bone turnover,[16] which can be distinguished by markers of bone formation, PICP, and intact osteocalcin.[31] Our data show that women carrying the Px haplotype have significantly higher mean PTH, and also tend to have higher levels of PICP and osteocalcin (albeit not significant). Women carrying both the Px haplotype and "s" allele have significantly the highest mean PTH ($P < .001$) and PICP ($P < .05$) levels, and a similar but nonsignificant trend for osteocalcin. In our sample, the women lacking the Px haplotype and carrying the "s" allele had a low mean PTH and the lowest mean PICP and osteocalcin levels (Fig 4). It would seem that allele "s" (at the Sp1 recognition locus), when present in women lacking the Px haplotype, impairs the transcription of the *COLIA1* gene, which results in lower levels of PICP. The opposite obtains in women carrying both the "s" allele and the Px haplotype. It has already been suggested that the *COLIA1*

Sp1 polymorphism is a functional genetic variant, and that the "s" allele increases binding affinity for the transcription factor Sp1.[32] This in turn leads to increased ratio of α1(I) protein relative to α2(I), as well as to reduced bone strength in "Ss" individuals as compared to "SS" ones. It has also been shown that in osteoblasts, estrogen regulates collagen type I levels mainly by the *ER*α isoform.[33] Our own data affirm the possible protein-protein interaction between ERα and Sp1, such as may regulate a gene transcription in line with that proposed in Klinge's[34] review article, and as has already been demonstrated for several genes.[35-38]

Possibly the above-suggested interaction might further involve the receptor pathways of any of the calciotropic hormones [PTH and $1,25(OH)_2D$], because the levels of PTH and 25(OH)D observed by us appeared to be unique for subjects with the di-loci genotype of Px haplotype and "s" allele, all of which resulted in altered expression of the protein product (the collagen type Iα1 polypeptide chain) and higher levels of PICP. However, the impact of these polymorphisms on the transcription and clinical end points has yet to be assessed.

If to recap, the current study indicates that elderly women carrying the Px haplotype in combination with the "s" allele are at greater risk of low BMD, which might partially be the result of high circulating levels of PTH. Admittedly, there are several potential limitations with this study. To begin with, it was performed on a modest sample size of 122 elderly women. Second, as the study population does not have access to modern medical services, nor to treatments such as hormone replacement therapy or calcium supplements, it is not possible to evaluate the contribution of potential covariates or to extrapolate the present results to the general modern population. Furthermore, the data from this study are useful in generating hypotheses, and these need to be tested experimentally. Finally, the fact that our group of women carrying both the Px haplotype and "s" allele was comprised of only 4 individuals implies that any results obtained by analyses performed with this minute group need to be considered with due caution. Yet, we should bear in mind that the group represented a combination of 2 relatively rare genotypes and consistently produced extreme mean values of PTH, PICP, osteocalcin, 25(OH)D, and BMD. We believe and hope that future studies would resort to combination of genotypes as a rule rather than an exception, and to be sure, further investigation is needed to ascertain whether our findings regarding this interlocus interaction effects on hormones, bone turnover markers and bone density in elderly women are reproducible also in other populations.

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REFERENCES

1. Grant SFA, Reid DM, Blake G, et al: Reduced bone density and osteoporosis associated with a polymorphic SP1 binding site in the collagen type I α1 gene. Nat Genet 14:203-205, 1996

2. Keen RW, Woodford-Richens KL, Grant SFA, et al: Association of polymorphism at the type I collagen (*COLIA1*) locus with reduced bone mineral density, increased fracture risk, and increased collagen turnover. Arthritis Rheum 42:285-290, 1999

3. Uitterlinden AG, Weel AEAM, Burger H, et al: Interaction between the vitamin D receptor gene and collagen type Iα1 gene in susceptibility for fracture. J Bone Miner Res 16:379-385, 2001

4. Brown MA, Haughton MA, Grant SFA, et al: Genetic control of bone density and turnover: role of the collagen Iα1, estrogen receptor, and vitamin D receptor genes. J Bone Miner Res 16:758-764, 2001

5. Kobayashi S, Inoue S, Hosoi T, et al: Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 11:306-311, 1996

6. Han KO, Moon IG, Kang YS, et al: Nonassociation of estrogen receptor genotypes with bone mineral density and estrogen responsiveness to hormone replacement therapy in Korean postmenopausal women. J Clin Endocrinol Metab 82:991-995, 1997

7. Becherini L, Gennari L, Masi L, et al: Evidence of linkage disequilibrium between polymorphisms in the human estrogen receptor α gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Genet 9:2043-2050, 2000

8. Albagha OME, McGuigan FEA, Reid DM, et al: Estrogen receptor α gene polymorphisms and bone mineral density: Haplotype analysis in women from the United Kingdom. J Bone Miner Res 16:128-134, 2001

9. Pacifici R: Cytokines, estrogen and postmenopausal osteoporosis—The second decade. Endocrinology 139:2659-2661, 1998

10. Gallagher JC, Kinyamu HK, Fowler SE, et al: Calciotropic hormones and bone makers in the elderly. J Bone Miner Res 13:475-482, 1998

11. Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, et al: Serum oestradiol and oestrogen-receptor gene polymorphism are associated with bone mineral density independently of serum testosterone in normal males. Clin Endocrinol 49:803-809, 1998

12. Sapir-Koren R, Livshits G, Landsman T, et al: Bone mineral density is associated with estrogen receptor gene polymorphism in men. Anthrop Anz 59:343-353, 2001

13. Sapir-Koren R, Livshits G, Kobyliansky E: Association and linkage analysis suggest genetic effects of estrogen receptor α and collagen IA1 genes on bone mineral density in caucasian women. Calcif Tissue Int (in press) Online First Publications (Feb 2003) doi: 10.1007/S00223-002-2006-5

14. Lips P, Duong T, Oleksik A, et al: A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: Baseline data from the multiple outcomes of raloxifene evaluation clinical trial. J Clin Endocrinol Metab 86:1212-1221, 2001

15. Gallagher LC: Patophysiology of osteoporosis. Semin Nephrol 12:109-115, 1992

16. Chapuy MC, Schott AM, Garnero P, et al: Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. J Clin Endocrinol Metab 81:1129-1133, 1996

17. Livshits G, Karasik D, Pavlovsky OM, et al: Segregation analysis reveals a major gene effect in compact and cancellous bone mineral density in two populations. Hum Biol 71:155-172, 1999

18. Livshits G, Karasik D, Kobyliansky E: Complex segregation analysis of the radiographic phalanges BMD and their age related changes. J Bone Miner Res 17:152-161, 2002

19. Livshits G, Pavlovsky OM, Kobyliansky E: Population biology of human aging: Segregation analysis of bone age characteristics. Hum Biol 68:539-544, 1996

20. Versluis R, Petri H, Vismans F, et al: The relationship between phalangeal bone density and vertebral deformities. Calcif Tissue Int 66:1-4, 2000

21. Livshits G, Yakovenko C, Kobyliansky E: Quantitative genetic

analysis of circulating levels of biochemical markers of bone formation. Am J Med Genet 94:324-331, 2000

22. Gallagher JC, Riggs CL, DeLuca HF: Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. J Clin Endocrinol Metab 51:1359-1364, 1980

23. Khosla S, Atiknson EJ, Melton J, et al: Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: A population-base study. J Clin Endocrinol Metab 82:1522-1527, 1997

24. Heshmati HM, Khosla S, Robins SP, et al: Role of low levels of endogenous estrogen in regulation of bone resorption in late postmenopausal women. J Bone Miner Res 17:172-178, 2002

25. Naveh-Many T, Almogi G, Livni N, et al: Estrogen receptors and biologic response in rat parathyroid tissue and C cells. J Clin Invest 90:2434-2438, 1992

26. Deng HW, Li J, Li JL, et al: Change of bone mass in postmenopausal Caucasian women with and without hormone replacement therapy is associated with vitamin D receptor and estrogen receptor genotypes. Hum Genet 103:576-585, 1998

27. Ongphiphadhanakul B, Chanprasertyothin S, Payatikul P, et al: Oestrogen-receptor-alpha gene polymorphism affects responses in bone mineral density to oestrogen in postmenopausal women. Clin Endocrinol (Oxf) 52:581-585, 2000

28. Matsubara Y, Murata M, Kawano K, et al: Genotype distribution of estrogen receptor polymorphisms in and postmenopausal women from healthy and coronary populations and its relation to serum lipid levels. Arterioscler Thromb Vasc Biol 17:3006-3112, 1997

29. Herrington DM, Howard TD, Hawkins GA, et al: Estrogen receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women coronary disease. N Engl J Med 346:967-974, 2002

30. Herrington DM, Howard TD, Brosnihan KB, et al: Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. Circulation 105:1879-1882, 2002

31. De Leo V, Ditto A, la Marca A, et al: Bone mineral density and biochemical markers of bone turnover in peri- and postmenopausal women. Calcif Tissue Int 66:263-267, 2000

32. Mann V, Hobson EE, Li B, et al: A *COLIA1* Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. J Clin Invest 107:899-907, 2001

33. Waters KM, Rickard DJ, Riggs BL, et al: Estrogen regulation of human osteoblast function is determined by the stage of differentiation and the estrogen receptor isoform. J Cell Biochem 83:448-462, 2001

34. Klinge CM: Estrogen receptor interaction with co-activators and co-repressors. Steroids 65:227-251, 2000

35. Krishnan V, Wang X, Safe S: Estrogen receptor-Sp1 complexes mediate estrogen-induced cathepsin D gene expression in MCF-7 human breast cancer cells. J Biol Chem 269:15912-15917, 1994

36. Porter W, Wang F, Wang W, et al: Role of estrogen receptor/Sp1 complexes in estrogen-induced heat shock protein 27 gene expression. Mol Endocrinol 10:1371-1378, 1996

37. Porter W, Saville B, Hoivic D, et al: Functional synergy between the transcription factor Sp1 and the estrogen receptor. Mol Endocrinol 11:1569-1580, 1997

38. Petz LN, Nardulli AM: Sp1 binding sites and an estrogen response element half-site are involved in regulation of the human progesterone receptor A promoter. Mol Endocrinol 14:972-985, 2000