Interaction Between Birthweight and Polymorphism in the Calcium-Sensing Receptor Gene in Determination of Adult Bone Mass: The Hertfordshire Cohort Study

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ABSTRACT. ***Objective.*** We sought evidence of interaction between single-nucleotide polymorphisms (SNP) in the calcium-sensing receptor (*CASR*) gene and early life in determination of bone mineral density (BMD) among individuals from the Hertfordshire Cohort Study.

Methods. Four hundred ninety-eight men and 468 women aged 59–71 years were recruited. A lifestyle questionnaire was administered and BMD at lumbar spine and femoral neck was measured. DNA was obtained from whole blood samples using standard extraction techniques. Five SNP of the *CASR* gene termed CASRV1 (rs1801725, G→T, S986A), CASRV2 (rs7614486, T→G, untranslated), CASRV3 (rs4300957, untranslated), CASRV4 (rs3804592 G→A, intron), and CASRV5 (rs1393189, T→C, intron) were analyzed.

Results. Among women the 11 genotype of the CASRV3 SNP was associated with higher lumbar spine BMD within the lowest birthweight tertile, while the opposite pattern was observed among individuals in the highest birthweight tertile (test for interaction on 1 df, $p = 0.005$, adjusted for age, body mass index, physical activity, dietary calcium intake, cigarette and alcohol consumption, social class, menopausal status, and hormone replacement therapy use). Similar relationships were seen at the total femur ($p = 0.042$, fully adjusted) with birthweight and at the total femur according to weight at 1 year tertile among women ($p < 0.001$, fully adjusted). One haplotype was associated with lumbar spine BMD in women ($p = 0.008$, fully adjusted); these findings were replicated in a second cohort.

Conclusion. We have found evidence of an interaction between a SNP of the *CASR* gene and birthweight in determination of bone mass in a UK female population. (First Release Feb 15 2007; J Rheumatol 2007;34:769–75)

Key Indexing Terms:
BONE BONE DENSITY COHORT STUDIES GENETIC STUDIES

Twin and family studies confirm an inherited contribution to peak bone mass, and various candidate genes have been proposed for the genetic regulation of bone mineral, including the genes for the vitamin D receptor, the estrogen receptor, and type I collagen (Col IA1). However, polymorphisms in these genetic loci explain only a small portion of the observed variance in bone mass in the general population[1-5]. Evidence is also accumulating that the risk of later osteoporosis might be programmed by environmental influences during intrauterine or early postnatal life. Growth in infancy, a marker of such programming, predicts adult bone mass independently of adult lifestyle[6-9]. We published evidence of such an interaction between polymorphisms of the growth hormone gene, birthweight, and adult bone mass in a cohort of Hertfordshire, UK, men and women[10,11]. However, little is known of the interaction between intrauterine environment and genetic markers of calcium (Ca) metabolism and their association with adult bone mineral density (BMD).

In vitro studies have demonstrated that chondrogenic and osteogenic function can be influenced in response to different extracellular Ca concentrations[12]. Heath, *et al* described a polymorphism in exon 7 of the Ca-sensing receptor (*CASR*) gene, coding the intracellular domain of the receptor[13]. This polymorphism, A986S, was found to be a predictor of circulating Ca concentrations in women[14,15]. However, to date, results relating this gene to BMD have been conflicting. We sought to evaluate the interaction between polymorphisms of the *CASR* gene and early life in the determination of BMD in a large well-characterized elderly cohort.

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MATERIALS AND METHODS

In our study, which was designed to examine the relationship between growth in infancy and the subsequent risk of osteoporosis, the selection procedure was as follows: in brief, with the help of the National Health Service Central Registry at Southport and Hertfordshire Family Health Service Association, we traced men and women born between 1931 and 1939 in Hertfordshire, who still lived in East Hertfordshire in 1998. With written permission from each subject's general practitioner, we approached each person by letter, asking if he or she would be willing to be contacted by one of our research nurses. If the subjects agreed, a research nurse performed a home visit and administered a structured questionnaire. This included information on socioeconomic status, medical history, drug history, cigarette smoking, alcohol consumption, and reproductive variables in women. A total of 768 men and 714 women completed the home visit; of this number, 737 men and 675 women subsequently attended clinic.

At clinic, height was measured to the nearest 0.1 cm using a Harpenden pocket stadiometer (Chasmors Ltd., London, UK) and weight to the nearest 0.1 kg on a Seca floor scale (Chasmors Ltd.). Venous whole blood samples were taken at this clinic visit.

Eligible subjects were then invited to reattend for bone density measurements. Individuals taking drugs known to alter bone metabolism (such as bisphosphonates) were excluded from this part of the study, although women taking hormone replacement therapy (HRT) were allowed to participate. There were no other exclusion criteria. BMD was measured in 498 men and 468 women, by dual energy radiographic absorptiometry at the lumbar spine and proximal femur (neck, total, intertrochanteric and trochanteric regions, Ward's triangle) using a Hologic QDR 4500 instrument (Vertec Scientific, Reading, UK). Measurement precision error, expressed as coefficient of variation, was 1.55% for lumbar spine BMD, 1.45% for total femur, and 1.83% for femoral neck BMD for the Hologic QDR 4500; these figures were scans on the same day, getting on and off the table between examinations. Short-term (2 mo) precision error for the QDR 4500 was less than 1% for both sites (manufacturer's figures).

Genomic DNA was extracted from whole blood samples according to standard procedures. Single-nucleotide variants in the *CASR* gene termed CASRV1 (rs1801725, G→T, S986A), CASRV2 (rs7614486, T→G, untranslated), CASRV3 (rs4300957, untranslated), CASRV4 (rs3804592 G→A, intron), and CASRV5 (rs1393189, T→C, intron) were analyzed.

The different alleles were termed 1 or 2, resulting in genotypes 11, 12, and 22. Genotypes 12 and 22 were combined in the event of a low frequency of genotype 22. Analyses were conducted separately for men and women using Stata 8. The relationship between each continuously distributed phenotype variable and each single-nucleotide polymorphism (SNP) was explored using both analysis of variance and linear regression models. Analyses were conducted with and without adjustment for age, body mass index (BMI), typical activity level, dietary calcium intake, smoking status, alcohol intake, current social class, and menopausal status and HRT use for women. Haplotypes were inferred using the Phase package[16].

The East and North Hertfordshire Ethical Committees granted ethical permission for the study. All participants gave written informed consent.

RESULTS

The characteristics of the study population at baseline are displayed in Table 1. The mean age of the men was 64.3 and the women 65.6 years. Thirty-four percent of the men and 62% of women had never smoked, while 52% of men (28% of women) were ex-smokers and 15% of men (10% of women) were current smokers. Four percent of men and 18% of women were non-drinkers, while 21% of men and 12% of women were moderate drinkers (11–21 and 8–14 units of alcohol/wk, respectively, 1 unit being a single glass of wine or a single measure of spirits).

Table 1. Summary characteristics of study participants. Data are mean (SD) unless stated otherwise.

Characteristic	Men (n = 498)	Women (n = 468)
Age, yrs	64.3 (2.5)	65.6 (2.5)
BMI, kg/m^2*	26.6 (1.1)	26.8 (1.2)
Alcohol consumption (units per week)**	10.0 (3.0, 22.5)	2.5 (0.5, 7.0)
Habitual activity, % ***	64.0 (14.8)	61.3 (14.9)
No. (%), current manual social class (IIIM-V)†	277 (55.6)	286 (61.1)
No. (%), current non-manual social class (I-IIIN)†	193 (38.8)	182 (38.9)
Lumbar spine BMD, g/cm^2††	1.08 (0.16)	0.96 (0.17)
Femoral neck BMD, g/cm^2††	0.85 (0.12)	0.76 (0.12)
Total femoral BMD, g/cm^2††	1.04 (0.13)	0.90 (0.13)

* Geometric mean (SD). ** Median and interquartile range among drinkers; 20 men and 86 women stated they do not drink alcohol. *** Standardized score range 0–100 derived from frequency of gardening, housework, climbing stairs, and carrying loads in a typical week. Higher scores indicate greater level of activity. † Social class was unclassified for 28 men. I-IIIN and IIIM-V denote classes 1–3 (non-manual), and 3 (manual) to 5, of the 1990 OPCS Standard Occupational Classification scheme for occupation and social class. Social class was identified on the basis of own current or most recent full-time occupation for men and never-married women, but on the basis of the husband's occupation for ever-married women. †† 1 man was not scanned at the lumbar spine; 3 men and 1 woman were not scanned at the hip. BMI: body mass index; BMD: bone mineral density.

The distribution frequencies of each genotype were as follows: CASRV1: 11 – 78.1%, 12/22 – 21.9%; CASRV2: 11 – 58%, 12 – 35.7%, 22 – 6.3%; CASRV3: 11 – 71.1%, 12 – 26.0%, 22 – 2.9%; CASRV4: 11 – 72.7%, 12 – 25.0%, 22 – 2.3%; CASRV5: 11 – 80.8%, 12/22 – 19.3%. Among men, the CASRV2 22 genotype was underrepresented, and the CASRV2 12 genotype overrepresented ($p = 0.008$); similarly, among men the CASRV3 22 genotype was underrepresented and the CASRV3 12 genotype overrepresented ($p = 0.02$). None of the other genotypes differed by sex. There was no evidence of departure from Hardy-Weinberg equilibrium. A total of 22.2% had at least one S allele of the A986S polymorphism (coded CASRV1). Taking into account the poor power of the recessive genotype (SS), the population for this gene was divided into 2 groups according to the presence or absence of the S allele.

There were no statistically significant direct associations between any of the CASR SNP and bone mass at the lumbar spine or femoral neck in the cohort as a whole, whether women taking HRT were excluded or not (Table 2). However, we also investigated the possible interaction between genotype and early environment as predictors of bone mass (Table 3). Among women, the 11 genotype of the CASRV3 SNP was associated with higher lumbar spine BMD within the lowest birthweight tertile, while this genotype was associated with lower lumbar spine BMD in the highest birthweight tertile (test for interaction $p = 0.005$, fully adjusted for age, social

Table 2. Lumbar spine, femoral neck, and total femoral BMD in relation to genotype.

	CASRV1				CASRV3					
	Men		Women		Men			Women		
Genotype	11	12/22	11	12/22	11	12	22	11	12	22
Lumbar spine BMD	1.07 (0.16)	1.09 (0.15)	0.96 (0.18)	0.95 (0.16)	1.08 (0.16)	1.06 (0.16)	1.05 (0.14)	0.96 (0.17)	0.94 (0.16)	1.05 (0.24)
n	376	110	356	96	338	136	8	322	104	19
p value adjusted	0.194		0.127			0.239			0.120	
unadjusted	0.226		0.506			0.174			0.614	
Femoral neck BMD	0.85 (0.12)	0.84 (0.13)	0.76 (0.12)	0.75 (0.11)	0.85 (0.12)	0.85 (0.13)	0.90 (0.12)	0.76 (0.12)	0.74 (0.12)	0.75 (0.15)
n	374	110	355	96	336	136	8	322	103	19
p value adjusted	0.598		0.083			0.530			0.889	
unadjusted	0.465		0.502			0.787			0.209	
Total femoral BMD	1.04 (0.13)	1.03 (0.14)	0.90 (0.13)	0.89 (0.13)	1.04 (0.13)	1.03 (0.14)	1.07 (0.13)	0.90 (0.13)	0.89 (0.12)	0.91 (0.17)
n	374	110	355	96	336	136	8	322	103	19
p value adjusted	0.538		0.593			0.625			0.890	
unadjusted	0.615		0.126			0.859			0.159	

Values are presented as means (SD). P values are given unadjusted on 1 df and adjusted for age at clinic, BMI, physical activity score, dietary calcium intake, smoking status, alcohol unit intake per week, current social class, years since menopause, HRT status. BMD: bone mineral density.

Table 3. Lumbar spine and total femoral BMD in women according to genotype and birthweight and weight at 1 year.

	Lumbar Spine BMD			Total Femoral BMD		
CASRV3	11	12	22	11	12	22
Birthweight (tertile)						
Low ≤ 3175 g	0.96 (0.18)	0.92 (0.16)	0.90 (0.13)	0.90 (0.14)	0.87 (0.12)	0.86 (0.09)
n	129	51	6	129	51	6
Middle 3175–3629 g	0.98 (0.17)	0.97 (0.17)	1.05 (0.29)	0.90 (0.14)	0.91 (0.13)	0.94 (0.23)
n	111	34	7	111	34	7
High > 3629 g	0.95 (0.17)	0.95 (0.16)	1.19 (0.20)	0.89 (0.12)	0.91 (0.10)	0.94 (0.14)
n	82	19	6	82	18	6
p for interaction unadjusted		0.009			0.046	
adjusted		0.005			0.042	
Weight at 1 year of age (tertile)						
Low ≤ 9185 g	0.95 (0.15)	0.94 (0.15)	0.90 (0.18)	0.90 (0.12)	0.87 (0.13)	0.85 (0.05)
n	95	48	3	95	48	3
Middle 9185–9979 g	0.98 (0.17)	0.89 (0.14)	1.08 (0.22)	0.91 (0.14)	0.87 (0.11)	0.91 (0.19)
n	100	26	10	100	26	10
High > 9979 g	0.96 (0.19)	0.98 (0.19)	1.08 (0.31)	0.88 (0.14)	0.93 (0.11)	0.95 (0.17)
n	127	30	6	127	29	6
p for interaction unadjusted		0.17			0.003	
adjusted		0.07			0.000	

Values are presented as means (SD). P values for interaction are given unadjusted and adjusted (on 1 df) for age at clinic, BMI, physical activity score, dietary calcium intake, smoking status, alcohol unit intake per week, current social class, years since menopause, HRT status. BMD: bone mineral density.

class, BMI, physical activity, Ca intake, cigarette and alcohol consumption, menopausal status, and HRT use). Similar relationships were observed at the total femur (test for interaction, fully adjusted p = 0.042). In addition, the 1 allele of the CASRV3 SNP was associated with higher total femoral BMD among women in the lowest weight at 1-year tertile (test for interaction, fully adjusted p < 0.001); in the highest weight at 1-year tertile the 1 allele was, again, associated with lower total femoral BMD (Figure 1). We expanded these analyses to utilize other cutpoints for early life measures (birth weight < 2500 g, 2500–3000 g, 3000–3500 g, 3500–4000 g, > 4000 g). While these revised groupings made little difference to our results, further interpretation was difficult due to the small numbers of women in the lowest birthweight group (11: 11 subjects; 12: 10 subjects; 22: 0 subjects). No significant genotype–early environment interactions were observed in men.

Six haplotypes of the 5 CASR SNP accounted for 92.3% of all the haplotypes defined in the Hertfordshire Cohort Study dataset: 11111, 11121, 12211, 21111, 11112, 12111. Significant associations were observed between the 12211 haplotype (the third most frequent in this population) and lum-

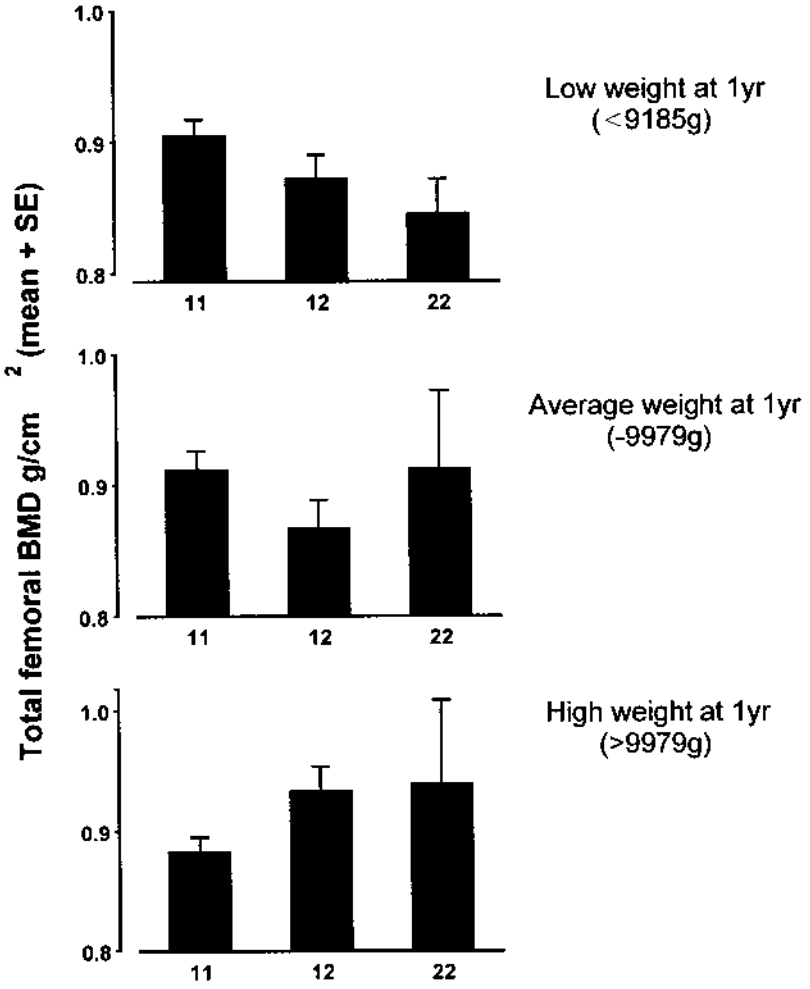

Figure 1. Total femoral BMD by CASRV3 genotype according to weight at 1 year of age in 444 women. See text for adjustments. P < 0.001 on 1 df.

Table 4. Associations of BMD with haplotypes of the CASR gene among men and women from the Hertfordshire Cohort Study.

	Haplotype	Men				Women			
		Frequency (n)	Mean Lumbar Spine BMD, g/cm^2	Mean Femoral Neck BMD, g/cm^2	Mean Total Femoral BMD, g/cm^2	Frequency (n)	Mean Lumbar Spine BMD, g/cm^2	Mean Femoral Neck BMD, g/cm^2	Mean Total Femoral BMD, g/cm^2
1	11111	0.43 (384)	1.05	0.85	1.04	0.46 (386)	0.97	0.76	0.90
2	11121	0.15 (129)	1.08	0.85	1.04	0.13 (108)	0.95	0.75	0.88
3	12211	0.15 (129)	1.03	0.85	1.03	0.11 (94)	1.00	0.76	0.91
4	21111	0.00 (0)	—	—	—	0.09 (76)	0.96	0.76	0.89
5	11112	0.11 (96)	1.07	0.84	1.04	0.06 (51)	0.96	0.79	0.92
6	12111	0.06 (55)	1.07	0.87	1.06	0.08 (70)	0.94	0.76	0.90
	All	445 men	1.07 ± 0.01	0.85 ± 0.01	1.04 ± 0.01	423 women	0.96 ± 0.01	0.76 ± 0.01	0.90 ± 0.01

BMD: bone mineral density.

bar spine BMD in women (Table 4; p = 0.008, adjusted for age at clinic, BMI, physical activity, dietary Ca score, social class, cigarette and alcohol consumption, years since menopause and HRT use in women). We sought to replicate our findings using an older community from the Hertfordshire Cohort that has been extensively described previously[10]. Indeed, our findings were replicated among this group of 186 men and 122 women aged 61–73 years at study, with the 12211 haplotype once again being associated with higher lumbar spine BMD among women (p = 0.05, fully adjusted).

DISCUSSION

We have demonstrated evidence of an interaction between a SNP of the *CASR* gene and early environment in the effect on BMD in a Hertfordshire population of healthy elderly women. To our knowledge, this is the first report relating CASR genotype to BMD taking into account the possible effects of development in early life. We have also demonstrated that one haplotype was associated with lumbar spine BMD in women, a finding replicated in a second population.

There were a number of limitations to this study. The individuals studied were selected because we have accurate records of their early life; these subjects were all born in Hertfordshire, and still live there. They have, however, previously been shown to have anthropometric and lifestyle characteristics similar to those of the general population[17]. The allelic frequency of the recessive alleles of both CASRV1 and CASRV3 polymorphisms was fairly low. However, the effect of the CASRV3 recessive genotype is consistent in BMD measurements at different sites and different ages, suggesting that our results are not a coincidental finding. Our genotype frequencies of the A986S (CASRV1) polymorphism, however, are comparable to those found by other groups.

We included SNP located in noncoding and intergenic regions, rather than focusing exclusively on the coding region. It is hypothesized that variations underlying complex diseases are not limited to the structure of the encoded protein. Gene regulation is the result of the combinatorial action of multiple transcription factors binding at multiple sites in and near a gene and it has recently been shown that gene expression regulatory elements reside in noncoding and intergenic regions[18].

Ca homeostasis has an important role in the regulation of bone remodeling, and alterations of the mechanisms involved in this regulation may contribute to the development of metabolic bone diseases. The main homeostatic regulator of extracellular Ca is the calcitropic parathyroid hormone (PTH). However, the *CASR* mediates this pathway through its ability to sense small changes in circulating Ca concentration and to activate intracellular pathways if its setpoint is not met. These pathways lead to an increase in PTH, which in turn will enhance the activation of vitamin D[19]. Both hormones increase circulating Ca by stimulating Ca resorption in renal tubules and intestine, and by stimulating bone resorption through promoting the differentiation of osteoclasts from multinucleated precursors[20,21].

The *CASR* is defective in individuals with familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism due to inactivating mutations[22,23]. Heath, *et al* suggested that the NH2-terminal extracellular and membrane-spanning regions of the receptor protein are functional domains for Ca binding and signal transduction, and mutations in these regions lead to familial benign hypocalciuric hypercalcemia[13].

An association between a Ca-repeat polymorphism of the *CASR* gene and BMD was found by Tsukamoto, *et al*[24]. Cole, *et al*[14,15] reported associations between the A986S polymorphism and Ca levels in a clinical trial of 163 White Canadian women. The AA genotype was associated with lower Ca levels, indicating a loss of function at the receptor level as in FHH. They suggested that this polymorphism would have a potential role as a predictor of disorders that affect bone and mineral metabolism. Lorentzon, *et al*[25] and Eckstein, *et al*[26] confirmed this effect of polymorphisms on circulating Ca concentrations and found associations with BMD. However, after correction for physical activity, the polymorphisms no longer had an effect on BMD. Eckstein, *et al* found the S allele to be overrepresented in a group of 80 Israelis with low BMD, although age at menarche was found to be the main predictor of BMD in their group. Several other groups have examined the relationship between extracellular Ca or BMD and the A986S polymorphism without finding a significant association. Takacs, *et al*[27] and Cetani, *et al*[28] studied the polymorphism in a homogenous postmenopausal White Hungarian and Italian, respectively, female population and confirmed the functionality of the polymorphism in circulating Ca levels, but could not confirm any influence of the polymorphism on BMD. Young, *et al*[29] and Bollerslev, *et al*[30] analyzed Ca data for, respectively, 102 New Zealand and 1252 Canadian postmenopausal women. Bollerslev, *et al* found no significance of the A986S polymorphism in association with serum Ca levels or with BMD. Most recently, Perez-Castrillon, *et al* failed to find an association of the A986S polymorphism with lumbar spine BMD in 48 hypertensive women[31].

In our large homogeneous study group we found the same allele frequencies of the A and S alleles as previously found by other groups. However, we could not find a direct effect of the CASR A986S polymorphism on BMD, and can confirm that the results of the latest studies also apply for our UK cohort. There are several possible explanations for this negative result. The *CASR* may not have a crucial role in the regulation of osteoblast function. However, we also studied 4 other polymorphisms and found an interaction between the CASRV3 (C→T, untranslated) polymorphism and development in early life in determination of BMD in women. We previously reported data suggesting that the intrauterine environment, using birthweight as a marker, and early life, using weight at 1 year of age as a marker, may modulate the relationship between the vitamin D receptor and growth hormone genes and adult BMD[10,11,32]. Therefore we hypothesized that the effect of polymorphisms of the *CASR* gene on BMD may be modulated by development in early life. Our study provides evidence of an interaction between the early environment and a genotypic locus such that individuals who carry the unfavorable, recessive genotype who also grew poorly in fetal life and infancy are susceptible to lower BMD, whereas the recessive genotype predisposes to higher BMD among individuals that grew better in early life.

Men have greater bone mineral content and area, indicating a larger skeletal size, and this is normally reflected in a

higher BMD in men compared with women[33]. However, when adequate correction for body size is performed (by calculation of bone mineral apparent density, for example), the apparent differences between the sexes are much reduced, or removed. However, there are also known to be sex differences in age-related changes in bone loss rates and bone strength. Hence, in one study, men had approximately 30% larger cross-sectional bone size compared with women at age 67–69 years[34]. At all sites, women had 2- to 5-fold reduction in bone mass with age compared to men, but had comparable increments in bone size. This was reflected in significantly worse bone strength measures with age in women. The sex hormones are likely to be important contributors to the sexual dimorphism we describe. The effects of the sex steroid receptors on skeletal regulation have been tested recently in murine models[35]; androgen receptor-deficient mice follow the male pattern of long bone development, but imitate females in bone density and trabecular bone, while loss of the α subtype of the estrogen receptor resulted in increased bone length in females but reduced bone length in males. Loss of either receptor resulted in increased osteoblast sensitivity to PTH. Our study suggested an interaction between birthweight and the *CASR* with regard to BMD among women, but not men. Our study group were some years past menopause and hence their BMD would reflect the peak bone mass attained and the rate of subsequent bone loss. It is possible that the interaction we describe may reflect an interaction between birthweight and bone loss. Although the data we present are cross-sectional, the cohort is being followed up, and we will be able to test for an interaction with bone loss rate shortly.

Our study shows, for the first time, evidence of an interaction between a polymorphism of the *CASR* gene and growth in early life in the determination of bone mass in later life in a UK female population. Further work is now indicated to replicate this finding.

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REFERENCES

1. Seeman E, Hopper JL, Bach LA, et al. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 1989;20:554-8.
2. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 1995;10:2017-22.
3. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest 1987;80:706-10.
4. Kelly PJ, Eisman JA, Sambrook PN. Interaction of genetic and environmental influences on peak bone density. Osteoporos Int 1990;1:56-60.
5. Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CC Jr. Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res 1991;6:561-7.
6. Cooper C, Cawley M, Bhalla A, et al. Childhood growth, physical activity, and peak bone mass in women. J Bone Miner Res 1995;10:940-7.
7. Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D. Growth in infancy and bone mass in later life. Ann Rheum Dis 1997;56:17-21.
8. Jones G, Riley M, Dwyer T. Maternal smoking during pregnancy, growth, and bone mass in prepubertal children. J Bone Miner Res 1999;14:146-51.
9. Antoniades L, MacGregor AJ, Andrew T, Spector TD. Association of birth weight with osteoporosis and osteoarthritis in adult twins. Rheumatology Oxford 2003;42:791-6.
10. Dennison EM, Syddall HE, Rodriguez S, Voropanov A, Day IN, Cooper C. Polymorphism in the growth hormone gene, weight in infancy, and adult bone mass. J Clin Endocrinol Metab 2004;89:4898-903.
11. Day IN, Chen XH, Gaunt TR, et al. Late life metabolic syndrome, early growth, and common polymorphism in the growth hormone and placental lactogen gene cluster. J Clin Endocrinol Metab 2004;89:5569-76.
12. Chang W, Tu C, Chen TH, et al. Expression and signal transduction of calcium-sensing receptors in cartilage and bone. Endocrinology 1999;140:5883-93.
13. Heath H III, Odelberg S, Jackson CE, et al. Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalciuric hypercalcemia suggest receptor functional domains. J Clin Endocrinol Metab 1996;81:1312-7.
14. Cole DE, Peltekova VD, Rubin LA, et al. A986S polymorphism of the calcium-sensing receptor and circulating calcium concentrations. Lancet 1999;353:112-5.
15. Cole DE, Vieth R, Trang HM, Wong BY, Hendy GN, Rubin LA. Association between total serum calcium and the A986S polymorphism of the calcium-sensing receptor gene. Mol Genet Metab 2001;72:168-74.
16. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978-89.
17. Syddall HE, Aihie Sayer A, Dennison EM, Martin HJ, Barker DJP, Cooper C. Cohort profile: The Hertfordshire Cohort Study. Int J Epidemiol 2005;34:1234-42.
18. Luque RM, Kineman RD, Park S, et al. Homologous and heterologous regulation of pituitary receptors for ghrelin and growth hormone-releasing hormone. Endocrinology 2004;145:3182-9.
19. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. Nature 1993;366:575-80.
20. Purroy J, Spurr NK. Molecular genetics of calcium sensing in bone cells. Hum Mol Genet 2002;11:2377-84.
21. Spurr NK. Genetics of calcium-sensing — regulation of calcium levels in the body. Curr Opin Pharmacol 2003;3:291-4.
22. Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B. Mutations in the human Ca(2+)-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 1993;75:1297-303.
23. Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J. Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. Nat Genet 1994;8:303-7.
24. Tsukamoto K, Orimo H, Hosoi T, et al. Association of bone mineral density with polymorphism of the human calcium-sensing receptor locus. Calcif Tissue Int 2000;66:181-3.

25. Lorentzon M, Lorentzon R, Lerner UH, Nordstrom P. Calcium sensing receptor gene polymorphism, circulating calcium concentrations and bone mineral density in healthy adolescent girls. Eur J Endocrinol 2001;144:257-61.
26. Eckstein M, Vered I, Ish-Shalom S, et al. Vitamin D and calcium-sensing receptor genotypes in men and premenopausal women with low bone mineral density. Isr Med Assoc J 2002;4:340-4.
27. Takacs I, Speer G, Bajnok E, Tabak A, Nagy Z, Horvath C. Lack of association between calcium-sensing receptor gene "A986S" polymorphism and bone mineral density in Hungarian postmenopausal women. Bone 2002;30:849-52.
28. Cetani F, Pardi E, Borsari S, et al. Calcium-sensing receptor gene polymorphism is not associated with bone mineral density in Italian postmenopausal women. Eur J Endocrinol 2003;148:603-7.
29. Young R, Wu F, Van de Water N, Ames R, Gamble G, Reid IR. Calcium sensing receptor gene A986S polymorphism and responsiveness to calcium supplementation in postmenopausal women. J Clin Endocrinol Metab 2003;88:697-700.
30. Bollerslev J, Wilson SG, Dick IM, Devine A, Dhaliwal SS, Prince RL. Calcium-sensing receptor gene polymorphism A986S does not predict serum calcium level, bone mineral density, calcaneal ultrasound indices, or fracture rate in a large cohort of elderly women. Calcif Tissue Int 2004;74:12-7.
31. Perez-Castrillon JL, Sanz A, Silva J, Justo I, Velasco E, Duenas A. Calcium-sensing receptor gene A986S polymorphism and bone mass in hypertensive women. Arch Med Res 2006;37:607-11.
32. Dennison EM, Arden NK, Keen RW, et al. Birthweight, vitamin D receptor genotype and the programming of osteoporosis. Paediatr Perinat Epidemiol 2001;15:211-9.
33. Tuck SP, Pearce MS, Rawlings DJ, Birrell FN, Parker L, Francis RM. Differences in bone mineral density and geometry in men and women: the Newcastle Thousand Families Study at 50 years old. Br J Radiol 2005;78:493-8.
34. Sigurdsson G, Aspelund T, Chang M, et al. Increasing sex difference in bone strength in old age: The Age, Gene/Environment Susceptibility-Reykjavik study (AGES-REYKJAVIK). Bone 2006;39:644-51.
35. Tozum TF, Oppenlander ME, Koh-Paige AJ, Robins DM, McCauley LK. Effects of sex steroid receptor specificity in the regulation of skeletal metabolism. Calcif Tissue Int 2004;75:60-70.