

## Original articles

# Birthweight, vitamin D receptor genotype and the programming of osteoporosis

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## Summary

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Studies of the association between polymorphisms of the gene for the vitamin D receptor (VDR) and adult bone mass have been inconsistent, pointing to the possibility that gene–environment interactions may vary in different populations. We have demonstrated previously an association between weight in infancy (a marker of the intrauterine and early post-natal environment) and each of adult bone mass and VDR genotype. We therefore sought to extend these observations in an elderly UK cohort and to investigate the possibility of an interaction between these genetic and early environmental markers of later osteoporosis risk. One hundred and sixty-five men and 126 women aged 61–73 years for whom birth records were available underwent bone mass measurements at baseline and follow-up 4 years later. Whole-blood samples were obtained, DNA extracted using standard techniques and polymorphisms in the VDR and collagen type I $\alpha$ 1 (Col IA1) genes identified. In the cohort as a whole, there were no significant associations between either birthweight or VDR genotype and bone mineral density (BMD) or bone loss rate at either site. However, the relationship between lumbar spine BMD and VDR genotype varied according to birthweight. Among individuals in the lowest third of birthweight, spine BMD was higher ( $P = 0.01$ ) in individuals of genotype 'BB' after adjustment for age, sex and weight at baseline. In contrast, spine BMD was reduced ( $P = 0.04$ ) in individuals of the same genotype who were in the highest third of the birthweight distribution. A significant ( $P = 0.02$ ) statistical interaction was also found between VDR genotype and birthweight as determinants of BMD. Similar but slightly weaker associations were seen between lumbar spine bone mineral content (BMC) and VDR genotype in the lowest birthweight tertile. When examining the relationship between Col1A1 genotype and bone mass, lumbar spine BMC was higher in individuals of genotype 'Ss' or 'ss' in the lowest birthweight tertile ( $P = 0.02$ ) after adjustment for age, sex and weight at baseline. These results suggest that genetic influences on adult bone size and mineral density may be modified by undernutrition *in utero*.

## Introduction

Twin and family studies confirm an inherited contribution to peak bone mass,<sup>1–4</sup> and various candidates have been proposed for the genetic regulation of bone mineral, including the genes for the vitamin D receptor (VDR) and for 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.<sup>5,6</sup> Evidence is also accumulating that the risk of later osteoporosis may be programmed by environmental influences during intrauterine or early post-natal life. Growth in infancy, a marker of such programming, predicts adult bone mass independently of adult lifestyle,<sup>7–9</sup> and this asso-

ciation may be mediated by programming of the growth hormone/insulin growth factor (IGF)-I and hypothalamic–pituitary–adrenal (HPA) axes.<sup>10,11</sup> As the intrauterine environment might interact with documented genetic markers of osteoporosis, we investigated the relationship between weight in infancy, polymorphism in the VDR gene and adult bone mass. Our preliminary findings in a small sample of British women<sup>12</sup> suggested that VDR genotype was statistically significantly associated with weight at 1 year of age, and that this genetic marker also showed a weak, non-significant association with bone mass. We report here a larger study, in which we aimed to extend these observations and sought evidence of an interaction between birthweight or weight in infancy (markers of the intrauterine or early post-natal environment) and polymorphisms in the genes for each of the VDR and Col IA1, as determinants of bone mass and prospectively determined, age-related bone loss.

## Patients and methods

We studied 165 men and 126 women aged 60–75 years who completed a longitudinal study of osteoporosis examining the relationship between growth in infancy and the subsequent risk of osteoporosis. The selection procedure for these individuals has been described in detail previously.<sup>13</sup> Briefly, we traced all men and women born in east Hertfordshire between 1920 and 1930 who were still resident in the county. Data on birthweight and weight at 1 year were obtained from preserved midwife and health visitor records. All the subjects attended two clinics 4 years apart, where a lifestyle questionnaire was administered on each occasion. This collected information on cigarette smoking and alcohol consumption, physical activity and dietary calcium intake.

Bone mineral density (BMD) was measured in each subject at baseline and follow-up 4 years later by dual-energy X-ray absorptiometry at the lumbar spine and proximal femur. Measurements in the baseline study were performed on a Hologic QDR 1000 instrument (Hologic, Waltham, MA, USA); this was replaced by a new QDR 4500 instrument for the follow-up study. In order to ensure comparability between readings, 23 subjects were invited to have two bone density scans at follow-up, one on each instrument. This permitted the calculation of a conversion algorithm that was linear across the bone mineral values studied.<sup>13</sup> From these measurements, we were able to derive annual

bone loss rates, both as absolute values and as a percentage of original bone density. Short-term *in vivo* measurement precision for the QDR 1000 instrument, expressed as a coefficient of variation, was 1.1% for lumbar spine BMD and 1.8% for femoral neck BMD; these figures were obtained by scanning six volunteers who were not part of the study undergoing five scans on the same day, getting on and off the table between examinations. Long-term (4-year) precision for the QDR 1000 using a spine phantom was <1%.

Genomic DNA was extracted from whole-blood samples according to standard procedures. VDR genotype was determined by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, using the restriction endonuclease BsmI.<sup>14–16</sup> Similarly, polymorphism in the Col IA1 gene was determined by the PCR and RFLP analysis, using the restriction endonuclease *MscI* to discriminate two alleles, S and s, which correspond to the presence of guanine nucleotide and thymidine, respectively, as the first bases in the Sp-1 binding site in the first intron of the gene.<sup>17,18</sup>

Normality of variables was assessed, and variables were transformed as required. The STATA statistical software package was used for the analyses. Analysis of variance and  $\chi^2$  tests for linear trend were used to explore the associations between polymorphisms in each gene and both BMD and bone loss rate at the lumbar spine and proximal femur. These tests were specified in the study protocol; thereafter, multiple linear regression was performed to adjust for the effects of adult lifestyle variables that influence bone density and bone loss.

In order to explore the association between catch-up/catch-down growth, each genetic marker and bone density, we used the approach described by Ong *et al.*<sup>19</sup> Cross-sectional standard deviation (SD) scores were calculated for each subject in the cohort, based on the mean and SD of birthweight and weight at 1 year. The SD scores for men and women were calculated separately. Subjects were classified as 'changers' if they showed a change in weight SD score of more than 0.67 SD units (a value deemed to be clinically significant). Subjects whose weight SD scores differed by <0.67 SD units were classified as 'non-changers'.

## Results

The characteristics of the study population at baseline are shown in Table 1. The mean age of the men studied

**Table 1.** Characteristics at baseline among 165 men and 126 women aged 60–75 years

	Men Mean (SD)	Women Mean (SD)
Age (years)	66.0 (3.2)	65.4 (2.6)
Body mass index (kg/m <sup>2</sup> )	26.8 (3.4)	26.7 (4.1)
Calcium intake (mg/day)	725 (260)	648 (213)
Outdoor walking (min/day)	100 (95)	84 (55)
Baseline BMD (g/cm <sup>2</sup> )		
Lumbar spine	1.1 (0.2)	0.9 (0.2)
Femoral neck	0.8 (0.1)	0.7 (0.1)
Total proximal femur	1.0 (0.2)	0.8 (0.1)
Bone loss rate (%/year) <sup>a</sup>		
Lumbar spine	-0.4 (1.4)	0.3 (1.2)
Femoral neck	0.2 (1.5)	1.1 (1.4)
Total proximal femur	0.2 (1.4)	1.3 (1.2)

<sup>a</sup>A positive figure implies bone loss over the follow-up period.

were 66 years and of the women 65 years. Their mean birthweights were 3.61 kg and 3.47 kg, respectively, and calcium intake was higher among men (714 mg daily) than among women (651 mg daily). Eighty-four per cent of the men and 40% of the women had ever smoked, and their median weekly alcohol consumption was six units.

In the cohort as a whole, there were no statistically significant associations between either birthweight or VDR genotype and baseline BMD at the lumbar spine or proximal femur. However, the relationship between

lumbar spine BMD and VDR genotype varied according to birthweight. Table 2 shows the mean values for lumbar spine bone mineral content (BMC) and BMD according to VDR genotype in each third of the birthweight distribution. The trend in bone density across VDR genotype is presented both before and after adjustment for weight at baseline. Among individuals in the lowest third of birthweight, spine BMD was higher in those with the BB genotype ( $P = 0.01$  after adjustment for age, sex and weight at baseline). In contrast, BMD was higher among subjects with the bb genotype who were heavier at birth ( $P = 0.04$ ). Similar trends were seen between lumbar spine BMC and VDR genotype in the low-birthweight group ( $P = 0.02$  after adjustment for age, sex and weight at baseline).

Formal testing for a statistical interaction between birthweight and VDR genotype as determinants of lumbar spine BMD revealed an interaction between these two risk factors that was statistically significant ( $P = 0.02$ ). Figure 1 shows that these opposing trends of lumbar spine BMD with VDR genotype among subjects of different birthweight were observed consistently among men and women. The observation that these trends were little affected by adjustment for adult weight suggests that the environmental effect modification occurs during intrauterine rather than post-natal, life. The three birthweight groups of either sex did not differ significantly with respect to adult body mass index, cigarette and alcohol consumption, physical activity or dietary calcium intake. Relationships between VDR genotype and BMC or BMD at the

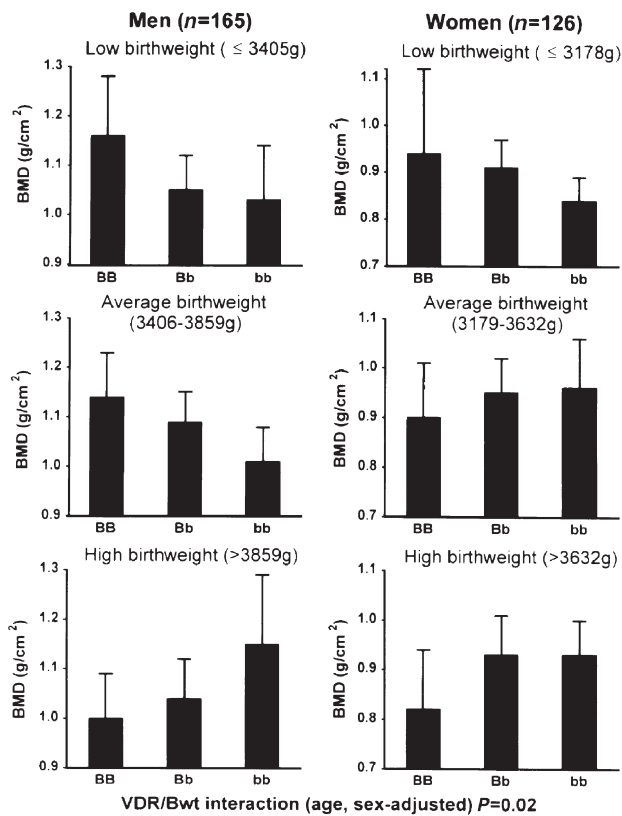
	VDR genotype	No. of subjects	Birthweight (thirds)		
			Low	Middle	High
BMD <sup>a</sup>	BB	61	1.07 (0.25)	1.03 (0.21)	0.93 (0.17)
	Bb	139	0.99 (0.18)	1.04 (0.17)	0.99 (0.18)
	bb	90	0.93 (0.19)	0.99 (0.17)	1.03 (0.21)
$P$ trend <sup>b</sup>			0.02	0.21	0.03
$P$ trend <sup>c</sup>			0.01	0.25	0.04
BMC <sup>a</sup>	BB	61	70.3 (22.3)	69.6 (20.8)	63.5 (18.5)
	Bb	139	63.7 (17.2)	70.4 (18.0)	65.3 (15.9)
	bb	90	58.8 (18.2)	66.2 (16.7)	67.7 (22.8)
$P$ trend <sup>b</sup>			0.05	0.17	0.07
$P$ trend <sup>c</sup>			0.02	0.21	0.08

**Table 2.** Birthweight, VDR genotype and lumbar spine bone mass in 290 Hertfordshire men and women aged 60–75 years

<sup>a</sup>Figures are mean lumbar spine BMD (g/cm<sup>2</sup>) or BMC (g) in each category, with SDs in parentheses.

<sup>b</sup> $P$ -value assessed as a trend variable after adjustment for age and sex.

<sup>c</sup> $P$ -value assessed as a trend variable after adjustment for sex, age and weight at baseline.



**Figure 1.** Lumbar spine BMD by VDR genotype according to birthweight tertile in 165 men and 126 women from a Hertfordshire cohort.

femoral neck showed similar patterns, with higher bone mass among those with the BB genotype who were born light, but with the bb genotype who were born in the heaviest third of the birthweight distribu-

tion (Table 3). The relationships were less pronounced, however, and the interaction between birthweight and VDR genotype as determinants of femoral neck bone mass was not significant.

We also explored the relationship between weight at 1 year, VDR genotype and adult bone mineral measurements. Although there were statistically significant positive associations between weight at 1 year and adult lumbar spine and femoral neck BMC ( $P < 0.03$ ), as described previously,<sup>8</sup> these were attenuated by adjustment for adult body weight or bone area. We did not observe a significant relationship between infant weight and VDR genotype in the cohort as a whole. Figure 2 also shows that there was no significant relationship between VDR genotype and lumbar spine BMD at any level of infant weight, and no apparent interaction between weight in infancy, VDR genotype and lumbar spine BMD. Findings for femoral neck BMD were similar.

Women lost bone at both sites; this ranged from 0.20% (SD 1.30%)/year at the lumbar spine to 1.05% (SD 1.54%)/year at the femoral neck (Table 1). In contrast, men lost only 0.36% (SD 2.10%)/year at the femoral neck and gained bone at the lumbar spine (0.30% [SD 1.59%]/year) over the 4-year period. We found no relationship between VDR genotype or birthweight and bone loss at either site.

In the analysis of polymorphism in the Col IA1 gene, there were only three men and three women with the ss genotype (Tables 4 and 5). The ss and Ss genotypes were therefore combined for statistical analyses. There

**Table 3.** Birthweight, VDR genotype and femoral neck bone mass in 291 Hertfordshire men and women aged 60–75 years

	VDR genotype	No. of subjects	Birthweight (thirds)		
			Low	Middle	High
BMD <sup>a</sup>	BB	61	0.77 (0.12)	0.80 (0.15)	0.75 (0.14)
	Bb	139	0.76 (0.13)	0.78 (0.11)	0.76 (0.13)
	bb	91	0.74 (0.13)	0.75 (0.14)	0.80 (0.13)
<i>P</i> trend <sup>b</sup>			0.79	0.06	0.08
<i>P</i> trend <sup>c</sup>			0.80	0.07	0.10
BMC <sup>a</sup>	BB	61	4.5 (0.9)	4.9 (1.2)	4.5 (1.0)
	Bb	139	4.4 (0.9)	4.8 (1.0)	4.5 (1.0)
	bb	91	4.2 (1.1)	4.6 (1.0)	4.7 (1.1)
<i>P</i> trend <sup>b</sup>			0.61	0.04	0.17
<i>P</i> trend <sup>c</sup>			0.59	0.04	0.22

<sup>a</sup>Figures are mean femoral neck BMD (g/cm<sup>2</sup>) as BMC (g) in each category, with SDs in parentheses.

<sup>b</sup>*P*-value assessed as a trend variable after adjustment for age and sex.

<sup>c</sup>*P*-value assessed as a trend variable after adjustment for sex, age and weight at baseline.

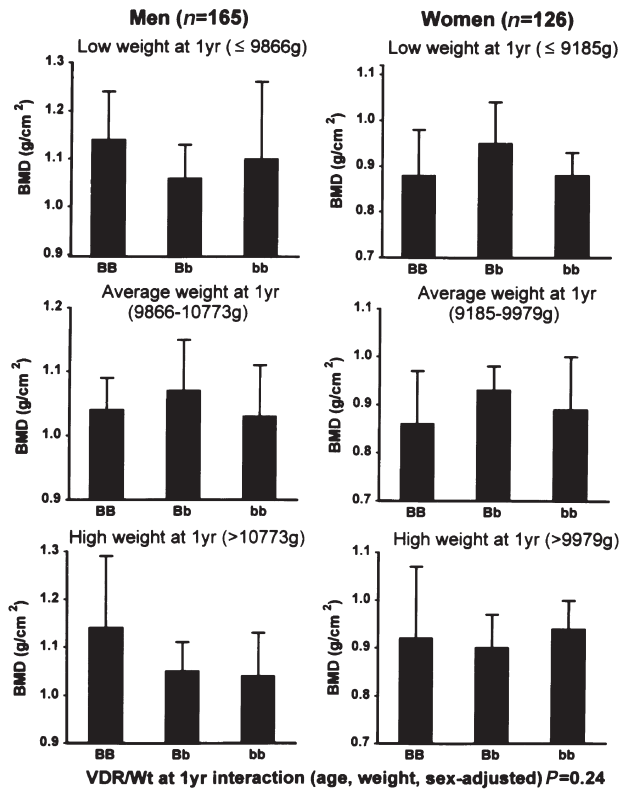


Figure 2. Lumbar spine BMD by VDR genotype according to thirds of weight at 1 year in 165 men and 126 women from a Hertfordshire cohort.

was no relationship between polymorphism in the Col IA1 gene and femoral neck BMC or BMD, nor was the polymorphism associated with bone loss rate at either the lumbar spine or the femoral neck. As in the case of polymorphism in the VDR gene, the addition of adult

environmental determinants of bone mass (physical activity, dietary calcium intake, cigarette smoking or alcohol consumption) did not appreciably alter the relationship between Col IA1 genotype and lumbar spine BMC. Lumbar spine BMC was significantly higher among individuals with the ss or Ss genotype in the lowest birthweight group, after adjusting for age, sex and body weight (Table 4). A similar pattern was observed for lumbar spine BMD, but the difference among low-birthweight individuals was not statistically significant. Consistent with these findings, we found that men and women with the s haplotype who were light at 1 year also had higher bone mass at the lumbar spine and femoral neck. Again, these relationships were not statistically significant ( $P = 0.06$ ); there was no significant interaction between weight at 1 year, Col IA1 genotype and adult BMC or BMD, and there was no significant effect of catch-up growth.

Of the 291 subjects, 132 (45.4%) were classified as 'non-changers', and the remaining 159 (54.6%) were classified as 'changers'.<sup>19</sup> The relation of mean lumbar spine bone mass with VDR genotype was similar in both 'changers' and 'non-changers', but the interaction between birthweight and VDR genotype as determinants of lumbar spine BMD was stronger among 'non-changers' ( $P = 0.09$ ) than among 'changers' ( $P = 0.42$ ).

## Discussion

We have explored the interaction between genetic and early environmental influences on bone density and age-related bone loss. We used two of the most widely

	Col IA1 genotype	No. of subjects	Birthweight (thirds)		
			Low	Middle	High
BMD <sup>a</sup>	SS	164	0.96 (0.20)	1.02 (0.17)	0.99 (0.20)
	Ss or ss	101	1.01 (0.20)	1.04 (0.19)	0.98 (0.16)
<i>P</i> trend <sup>b</sup>			0.09	0.52	0.66
<i>P</i> trend <sup>c</sup>			0.07	0.42	0.69
BMC <sup>a</sup>	SS	164	61.2 (18.5)	68.9 (18.2)	65.4 (19.5)
	Ss or ss	101	66.3 (19.1)	69.5 (18.9)	64.9 (17.3)
<i>P</i> trend <sup>b</sup>			0.04	0.50	0.99
<i>P</i> trend <sup>c</sup>			0.02	0.37	0.95

Table 4. Birthweight, Col IA1 genotype and lumbar spine bone mass in 265 Hertfordshire men and women aged 60–75 years

<sup>a</sup>Figures are mean lumbar spine BMD (g/cm<sup>2</sup>) or BMC (g) in each category, with SDs in parentheses.

<sup>b</sup>*P*-value assessed on 1 degree of freedom with adjustment for age and sex.

<sup>c</sup>*P*-value assessed on 1 degree of freedom with adjustment for age, sex and weight at baseline.

**Table 5.** Birthweight, Col IA1 genotype and femoral neck bone mass in 264 Hertfordshire men and women aged 60–75 years

	Col IA1 genotype	No. of subjects	Birthweight (thirds)		
			Low	Middle	High
BMD <sup>a</sup>	SS	163	0.75 (0.14)	0.78 (0.11)	0.77 (0.13)
	Ss or ss	101	0.75 (0.10)	0.77 (0.14)	0.77 (0.14)
<i>p</i> trend <sup>b</sup>			0.70	0.65	0.83
<i>p</i> trend <sup>c</sup>			0.72	0.72	0.70
BMC <sup>a</sup>	SS	163	4.3 (1.0)	4.8 (1.0)	4.5 (1.0)
	Ss or ss	101	4.4 (0.9)	4.7 (1.2)	4.6 (1.0)
<i>p</i> trend <sup>b</sup>			0.45	0.92	0.47
<i>p</i> trend <sup>c</sup>			0.43	0.76	0.37

<sup>a</sup>Figures are mean femoral neck BMD (g/cm<sup>2</sup>) or BMC (g) in each category, with SDs in parentheses.

<sup>b</sup>*P*-value assessed on 1 degree of freedom adjusted for age and sex.

<sup>c</sup>*P*-value assessed on 1 degree of freedom with adjustment for age, sex and weight at baseline.

studied genotypic markers of osteoporosis (polymorphism in the genes for the VDR<sup>14–16</sup> and in the Sp1 binding region of Col IA1),<sup>17,18,20</sup> and an epidemiological marker for an adverse intrauterine environment (low birthweight).<sup>21</sup> Our results suggest a statistically significant interaction between VDR genotype and birthweight as determinants of lumbar spine bone density, such that subjects with the bb genotype who are born light tend to have low bone density, whereas those with the same genotype born heavy have higher bone density. A similar, although statistically less robust, pattern was observed for the association between polymorphism in the Col IA1 gene and lumbar spine bone mass. For both candidate gene polymorphisms, evidence of interaction was strongest with birthweight and was not apparent for weight in infancy or for the rate of catch-up growth during the first year of life. Neither genetic marker was associated with prospectively determined bone loss rate in this cohort over a 4-year period, suggesting that these genetic influences act principally on the peak bone size and mineral density accrued during the first two decades of life, rather than on the metabolic pathways that control involuntional bone loss.

Evidence has now accumulated that the risk of osteoporosis may be modified by environmental influences during early life. Initial reports suggested that infants who were light at age 1 year had lower adult BMC at the lumbar spine and proximal femur.<sup>7–9,22</sup> These relationships have been documented in young adulthood, as well as in cohorts aged 60–70 years, where fracture incidence rates are substantially higher. More recently,

studies have demonstrated that birthweight is also a predictor of adult bone mass. Thus, birthweight was significantly correlated with spine and hip BMC in a US cohort of 305 women aged around 70 years.<sup>23</sup> Likewise, a British study<sup>24</sup> reported significant associations between birthweight and whole-body, spine and hip BMC among 143 men and women aged 70–75 years who were born and were still resident in Sheffield.

These observations are consistent with those of an Australian cohort study,<sup>25</sup> in which birthweight was found to predict whole-body bone mass at age 8 years. They also accord with follow-up studies of premature infants,<sup>26</sup> who appear to have deficits in bone size and mineral content during later childhood. Finally, the trends we observed between VDR genotype, birthweight and adult BMD were little changed by adjusting for adult body weight. As argued by Lucas *et al.*,<sup>27</sup> this finding suggests effect modification during prenatal, rather than post-natal, life. Taken together, these data suggest that environmental influences acting during intrauterine life explain, at least in part, the previously observed associations between weight at 1 year and adult bone mass.

Despite an initial report that polymorphisms in the VDR gene were associated with bone mass in twins,<sup>28</sup> other studies have yielded negative results. A recent meta-analysis that incorporated 16 studies<sup>29</sup> showed that individuals with the BB genotype (representing the absence of a restriction site) had lower bone density than those with the bb genotype. Potential explanations have included linkage disequilibrium between this VDR locus and the true genetic marker of mineralisa-

tion, as well as population admixture. Our findings confirm this observation, but only among subjects who were heavier at birth. They suggest, if anything, a stronger trend in the opposite direction among individuals who were light at birth. The data are consistent with an interaction between VDR genotype and an adverse intrauterine environment in the determination of adult bone density. Among individuals with a low calcium intake, the BB genotype is associated with a lower fractional calcium absorption.<sup>30</sup>

A previous twin study has also suggested a negative association between birthweight and calcium absorption.<sup>31</sup> Our observations among high-birthweight subjects, in whom the BB genotype was associated with lower BMD, are consistent with the hypothesis that intrauterine calcium availability modulates bone mineral accrual. This mechanism cannot explain the observation of a significant relationship between VDR genotype and bone density in the opposite direction, among subjects of low birthweight. Further research to unravel the interactions between a host of environmental influences and various candidate genes for osteoporosis is now required. Finally, the more pronounced findings at the lumbar spine compared with the proximal femur suggest that the greatest effect of VDR gene status on BMD is found in trabecular rather than cortical bone. However, this finding must be interpreted with caution, as it is at variance with the only published meta-analysis examining the relationship between VDR genotype and bone density.<sup>29</sup>

A French cohort study suggested that VDR gene polymorphism predicted sex-dependent growth during the first 2 years of life.<sup>32</sup> Among the 589 healthy infants studied, the girls who were homozygous for the BB polymorphism had higher length, weight and body surface area at 2 years of age than their bb counterparts. In contrast, the boys with the BB genotype were lighter and had lower body surface area. We did not find a statistically significant relationship between VDR gene polymorphism and birthweight in our population. However, in a pattern consistent with that observed in the French study, men homozygous for the BB polymorphism were lighter at age 1 year (3.61 kg) than those with the bb haplotype (3.72 kg). The relationship was reversed among women (BB weight at 1 year 3.47 kg; bb weight at 1 year 3.38 kg). Taken together, these data support the notion that VDR genotype may influence early post-natal growth through interaction with gender-related growth-regulatory genes, such as those coding the oestrogen receptor.<sup>33</sup>

The gene encoding Col IA1 is also an important candidate for the genetic regulation of bone density.<sup>6</sup> Mutations that affect the coding region of this gene are known to cause osteogenesis imperfecta. A polymorphism has recently been identified at a binding site for the transcription factor Sp1 in the Col IA1 gene<sup>17</sup> that is associated with bone mass and osteoporotic fracture.<sup>17,18,20</sup> It appears that this polymorphism is associated with differences in the affinity of Sp1 binding to DNA and with differences in the abundance of RNA transcripts derived from different alleles *in vivo* (with the s allele a marker of low bone density). However, the increased fracture risk observed with this allele persists after adjustment for bone density,<sup>17</sup> suggesting that it may be a marker of poor bone quality. The low frequency of the s haplotype in the British population limited our ability to explore this genetic marker with sufficient statistical power, but we found a weak association between lumbar spine BMC and Col IA1 genotype in the lowest birthweight group, such that individuals carrying the s allele had higher bone mass. The pattern observed more consistently in previous large population studies (where the s allele is associated with lower bone density) was seen among subjects of high birthweight. A more recent, population-based cohort study from Northern Ireland<sup>34</sup> has reported that subjects carrying the s allele tended to have low birthweight but normal adult height, again pointing to the possibility that birthweight modulates the relationship between Col IA1 genotype and adult bone size.

Our study was relatively small, and the observations clearly need to be replicated in other samples. It was, however, based on a representative British sample for whom unique neonatal anthropometry had been recorded many decades ago, and in whom both bone density and bone loss rates had been measured. We conclude that the intrauterine environment, as assessed by birthweight, may modulate the relationship between two commonly studied candidate gene polymorphisms for osteoporosis and adult bone size and density. Further studies to confirm this finding and to identify the specific environmental correlates of birthweight that might influence gene expression are now required.

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