

# Polymorphisms of the Vitamin D Receptor, Infant Growth, and Adult Bone Mass

R. W. Keen,<sup>1</sup> P. Egger,<sup>2</sup> C. Fall,<sup>2</sup> P. J. Major,<sup>1</sup> J. S. Lanchbury,<sup>3</sup> T. D. Spector,<sup>1</sup> C. Cooper<sup>2</sup>

<sup>1</sup>Rheumatology Department, St. Thomas' Hospital, London SE1 7EH, UK

<sup>2</sup>MRC Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK

<sup>3</sup>Molecular Immunogenetics Unit, UMDS, Guy's Hospital, London SE1 9RT, UK

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**Abstract.** Family and twin studies have demonstrated a strong genetic component to the development of peak bone mass. Early fetal and infant environment has also been shown to influence bone mass through an effect on skeletal size and mineral content. We report a retrospective study that has examined whether early infant growth is regulated by genetic factors shown to be associated with bone mass. We have determined the vitamin D receptor (VDR) gene alleles for 66 women (mean age 65.5 years) on whom detailed birth records were available. There was a statistically significant trend ( $P = 0.04$ ) for VDR genotype against weight at the age of 1 year, with the 'tt' homozygote group having 7% higher weight. We conclude that early fetal or infant environment may interact with an individual's underlying genotype to program early skeletal growth, and that this may track through later life to influence adult characteristics. Further prospective studies are required, however, to fully clarify the precise environmental and genetic mechanisms underlying these findings.

**Key words:** Bone density — Vitamin D receptor — Polymorphism — Growth — Genetic.

Osteoporosis is a systemic skeletal disease characterized by low bone mass, disorganization of bone microarchitecture, and an increased risk of fragility fracture. There is evidence that fetal and early infant environment can affect the incidence of several chronic adult diseases [1], and recent studies have demonstrated that early infant growth is correlated with young adult skeletal size and bone mineral content (BMC) [2, 3]. This relationship between measures of fetal and infant growth and adult bone mass may reflect an interaction between genetic and environmental factors in early life, which program the skeletal growth trajectory. Allelic variation in the vitamin D receptor (VDR) gene has been shown to influence bone mineral density (BMD) in some [4, 5], but not all populations [6, 7], and the aim of this study was to explore whether these polymorphisms had an important role in the regulation of early skeletal growth, and if this control might extend into later life when fractures become more frequent.

## Methods

From 1911 to 1948, the attending midwife notified families of each

birth in Hertfordshire and the birth weight was recorded. Health visitors also saw each child routinely during infancy and recorded its weight at the age of 1 year. With the help of the NHS Central Registry, men and women born during the period 1923–1930 have been traced, and details of this cohort have been previously described [8]. A group of 201 women aged 63–73 years had bone density measured at the lumbar spine (L1–L4) and femoral neck by dual energy X-ray absorptiometry (DXA) using a Hologic QDR-1000. Measurement precision, expressed as the coefficient of variation (cv), was 1.1% for the lumbar spine BMD and 1.8% for femoral neck BMD. Information was obtained on medical, gynecological and social history, calcium intake, activity, smoking, and alcohol consumption. Records were made of current height and weight. Blood samples were taken and stored in EDTA at  $-20^{\circ}\text{C}$  prior to DNA extraction.

Fifteen women were randomly selected from within each quintile of the distribution for weight at age 1 year for each sex, giving a total group size of 75 female subjects. DNA was extracted from the frozen blood samples on these subjects using standard techniques and PCR methods used to amplify a 740 bp fragment of the VDR gene [6]. The PCR product was digested with the restriction enzyme (*Taq I*) and alleles coded as 'T' (absence of restriction site) and 't' (presence of site). This gives rise to three genotypes—TT, Tt, and tt, where the genotype 'tt' is equivalent to the previously reported and low bone density genotype 'BB'. Statistical analysis was performed using an SAS personal computer (SAS Institute, Inc., Cary, NC).

## Results

Full vitamin D receptor (VDR) genotype results were available on 65 women (87%). Reasons for incomplete genotype data included failure of DNA extraction and unsuccessful PCR assay. The subjects with genotype results, however, did not differ significantly with regard to their baseline adult or infant characteristics from those without full genotype data. VDR genotypes (TT 38.5%, Tt 41.5%, tt 20%) and allelic frequencies ('T' = 0.59, 't' = 0.41) were not significantly different from those reported from other Caucasian populations [4, 5], and analysis of the genotype frequencies showed that Hardy-Weinberg equilibrium was maintained in this study population, suggesting no selection bias.

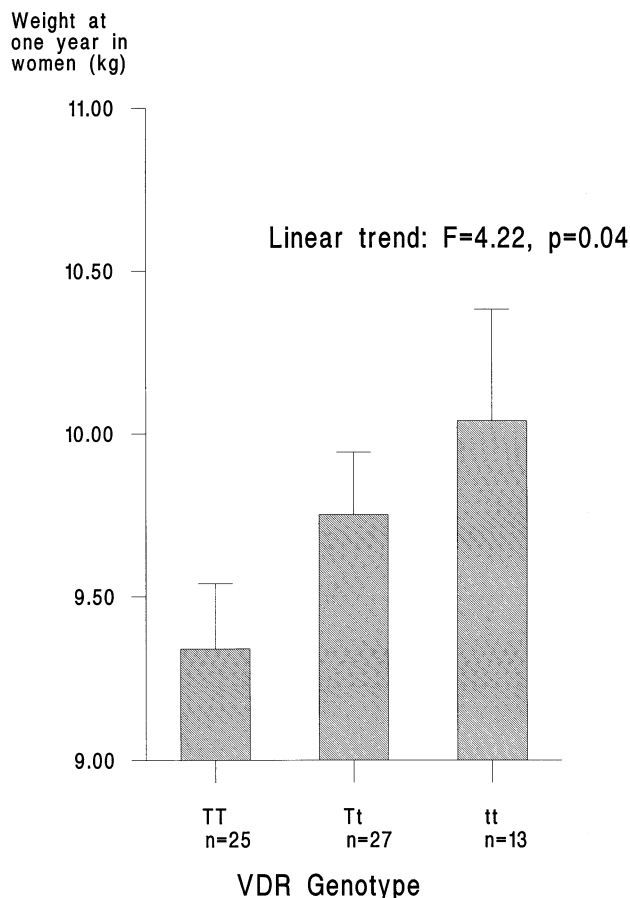
Baseline characteristics of the female group are shown in Table 1. As previous work suggested a codominant effect of VDR genotype on BMD [4, 5], we tested for association between infant weight and VDR genotype using a test for trend assuming the heterozygote Tt genotype would have an intermediate weight at 1 year of age compared with the homozygotes tt and TT. VDR genotype was significantly associated with weight at 1 year in female subjects ( $P = 0.04$ ) (Fig. 1), with a 7% difference in weight between the

**Table 1.** Characteristics of women (mean, 95% confidence interval)

Variable	VDR genotype		
	TT (n = 25)	Tt (n = 27)	tt (n = 13)
Age (years) <sup>a</sup>	64.88 (2.80)	65.30 (3.29)	67.00 (2.27)
Adult height (cm)	161 (159–163)	161 (159–163)	163 (160–167)
Adult weight (kg)	69.9 (64.5–75.2)	69.9 (66.9–72.9)	70.1 (64.4–75.8)
Birth weight (kg)	3.46 (3.26–3.66)	3.52 (3.32–3.69)	3.60 (3.35–3.88)
Weight at age 1 (kg)	9.34 (8.95–9.73)	9.75 (9.37–10.13)	10.04 (9.37–10.71)
LS BMD (g/cm <sup>2</sup> )	0.88 (0.82–0.94)	0.95 (0.89–1.01)	0.96 (0.86–1.07)
FN BMD (g/cm <sup>2</sup> )	0.73 (0.69–0.76)	0.71 (0.67–0.74)	0.73 (0.67–0.80)

LS = lumbar spine; FN = femoral neck

<sup>a</sup>Mean (SD)



**Fig. 1.** Female infant weight at 1 year of age (mean ± SEM) and VDR genotype.

homozygote genotypes. No significant effect of VDR genotype was seen on adult BMD or BMC at either the lumbar spine or femoral neck, or on adult height and weight.

## Discussion

Much attention is currently focused on clarifying the molecular and pathophysiological processes that underlie the development of osteoporosis. Family and twin studies have

both demonstrated a strong genetic component to BMD with an appreciable proportion of population variance attributable to specific genetic factors [9, 10]. The VDR gene has been proposed as a candidate gene for osteoporosis [4], although conflicting results have been obtained regarding its association with both baseline BMD and bone turnover [5–7]. Despite the recent retraction of the Australian twin data findings [4], there still appears to be a VDR genotype effect on BMD within certain population groups although the magnitude of this effect is much smaller than that originally reported.

The finding that early infant growth has an effect on adult skeletal size and BMC raises the possibility that genetic factors may also be regulating the early growth trajectory and that this tracks into a later effect on the adult skeleton [2]. Our data show a significant association between VDR genotype and female infant weight at 1 year. This suggests that early infant growth may be regulated in part by mechanisms that are mediated through vitamin D and its receptor. Other recent studies have also reported an association between skeletal size and VDR genotype. Specker et al. [11] prospectively studied 93 infants from the age of 3 to 12 months. No differences between VDR genotypes were seen regarding height or weight at either time point. There was, however, a difference in weight-corrected total body BMC at 12 months, with the bb (TT) homozygote having the lower value. Kelly et al. [12], however, failed to demonstrate any VDR genotype effect on birth weight or neonatal BMC in 28 neonates. A small study of 32 premenopausal women (mean age 36.9 years) demonstrated a VDR genotype association with weight and total body bone mineral [13], although the bb (TT) homozygote group was heavier and had greater bone mass. In a further study of 93 women (mean age 43.5 years) from the same group, the b (T) allele was associated with increased body weight and an increased rate of growth of hip bone dimensions over a 24-year study period [14].

The genetic association in our study did not track into adulthood as an effect on weight or adult BMD, although this may reflect the small sample size (n = 66). Previous work from the total female cohort has shown a positive correlation between infant weight and adult spine and hip BMC [3], although again, no VDR genotypic effect was seen on adult BMD or BMC at either the spine or hip. Larger studies of 200–300 participants may be required, however, to have sufficient power to detect a relationship between VDR genotype and adult BMD [15].

In our study, the direction of the VDR genotype effect on

weight at 1 year is the reverse of that expected from previous UK bone density reports [6]. We have observed that the "t" allele was associated with a statistically higher weight at 1 year, and a nonsignificant increase in adult weight and lumbar spine BMD. This difference in direction of the genotype effect might be explained in two ways. First specific environmental agents may interact differentially with the VDR locus as physiological studies have demonstrated differing bone response, calcium absorption, and response to calcitriol between VDR genotypes [16–18], effects that appear more marked in relative calcium-deficient states. Alternatively, the *TaqI* polymorphism may be in linkage disequilibrium with a gene influencing early growth and BMD located nearby on chromosome 12. The degree of linkage between the *TaqI* marker and this novel "growth gene" might then vary in different populations studied. Potential candidates for this locus include the insulin-like growth factor 1 (IGF-1) and *HoxC5* genes both of which map to the long arm of chromosome 12.

In conclusion, this study has shown a significant association between female infant weight and a *TaqI* polymorphism within the VDR gene. Further work is required to clarify whether this polymorphism is merely a genetic marker for early growth, or whether the VDR locus and the vitamin D/parathyroid hormone endocrine axis have a direct effect on programming the infant growth trajectory. Prospective studies will be required to examine specific environmental-gene interactions and their effect on subsequent growth and future disease incidence. Knowledge of these factors may lead to the development of novel preventative strategies for both osteoporosis and other skeletal conditions.

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