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Polymorphism in the Growth Hormone Gene, Weight in Infancy, and Adult Bone Mass

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Epidemiological studies point to the importance of gene-environment interactions during early life as determinants of later osteoporosis and fracture. We examined associations between common single nucleotide polymorphisms in the human GH (*GH1*) gene and weight in infancy, adult bone mass and bone loss rates, and circulating GH profiles. Two hundred and five men and 132 women, aged 61–73 yr, in the Hertfordshire Cohort Study were included; bone mineral density was measured by dual energy x-ray absorptiometry over 4 yr. Twenty-four-hour circulating GH profiles were constructed in a subset of 71 men and women. Genomic DNA was examined for two single nucleotide polymorphisms in the GH gene (one in the promoter region and one in intron 4). Homozygotes at loci *GH1* A5157G and T6331A displayed low baseline bone density and accelerated bone loss; there was also a significant ($P = 0.04$) interaction among weight at 1 yr, *GH1* genotype, and bone loss rate. There was a graded association between alleles and circulating GH concentration among men. This study suggests that common diversity in the *GH1* region predisposes to osteoporosis via effects on the level of GH expression. The interaction with infant weight suggests that early environment may influence the effect of *GH1* genotype on bone loss. (*J Clin Endocrinol Metab* 89: 4898–4903, 2004)

TWIN AND FAMILY studies confirm an inherited contribution to peak bone mass (1–4), and various candidates have been proposed for the genetic regulation of bone mineral, including the genes for the vitamin D receptor (*VDR*), the estrogen receptor, and type I collagen (*Col IA1*). However, polymorphisms in these genetic loci explain only a small proportion of the observed variance in bone mass in the general population (5–7). Evidence is also accumulating that the risk of later osteoporosis might be programmed by environmental influences during intrauterine or early postnatal life. Weight in infancy, a marker of such programming, predicts adult bone mass independently of adult lifestyle (8–10), and this association may be mediated by programming of the GH/IGF-I axis (11). However, little is known of the interaction between the intrauterine environment and genetic markers of GH activity. Such interactions may be explored using birth weight and weight in infancy as markers of the intrauterine and early postnatal environment. For example, we have recently published evidence of an interaction between the *VDR* genotype and birth weight in a cohort of Hertfordshire men and women (12). As intrauterine and early postnatal growth are under combined genetic and environmental regulation, and the effects of GH on linear growth manifest during infancy, we report a series of studies testing the hypotheses that 1) common single nucleotide polymorphisms (SNPs) in the GH gene cluster relate to adult bone mass; 2) these polymorphisms influence the activity of the GH axis in adult life; and 3) birth weight and infant weight might interact with these relationships. These SNPs were selected because they have been shown to be associated not only with disease in adults (13), but also with gene expression in *in vitro* studies (14).

Subjects and Methods

We studied 205 men and 132 women, aged 61–73 yr, who completed a longitudinal study of osteoporosis examining the relationship between growth in infancy and the subsequent risk of osteoporosis (the Hertfordshire Cohort Study). The selection procedure for these individuals has been described in detail previously (15). In brief, we traced all men and women born in East Hertfordshire, UK, between 1920–1930, who were still resident in the county. Birth weight and weight at 1 yr were obtained retrospectively from midwife and health visitor archived records. All subjects who participated in this study attended two clinics, 4 yr apart. At the first clinic, a lifestyle questionnaire was administered. 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 yr later by dual energy x-ray absorptiometry at the lumbar spine and proximal femur (15). From these measurements we were able to derive annual bone loss rates both as absolute values and as a percentage of the original bone density. Short-term *in vivo* measurement precision for the Hologic QDR 1000 (Vertec Scientific, Ltd., Calleva Park, Reading, UK), expressed as the 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. The long-term (4-yr) precision for the QDR 1000 using a spine phantom was less than 1%. Thoracolumbar radiographs taken at baseline were assessed for osteoarthritis according to the Kellgren and Lawrence score grading system (16).

Genomic DNA was extracted from 5 ml potassium-EDTA venous blood, quantified by picoGreen assay, and concentrations were equalized. Long-term stock DNA aliquots were made, and working 96-well plates of DNA dilutions to 7 ng/μl were prepared. Degenerate oligo primer amplifications were made from dilution plates to conserve stock

Abbreviations: BMD, Bone mineral density; CV, coefficients of variation; LD, linkage disequilibrium; NTX/Cr, type I collagen cross-linked N-telopeptide; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

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DNA, and 96- or 384-well PCRs were performed from degenerate oligo primer-DNA, representing 0.07 ng of the original genomic DNA. A 384-well, allele-specific PCR (using flanking primers and internal allele-specific primers) in conjunction with a 384-well, microplate array, diagonal gel electrophoresis; fluorescent image screening; and PhoretiX gel image analysis were used for SNP analyses (17). Two single nucleotide variants in the GH gene (coded GHV1 and GHV4) were analyzed. GHV1 was in the promoter region of the GH gene, whereas GHV4 lay in the fourth exon downstream of this. GHV1 represents an adenine to guanine translocation at position 5157 in GenBank sequence J03071; GHV4 is located in intron 4 of the GH gene at position 6331 in GenBank sequence J03071 and represents a thymidine to adenine transversion.

To characterize the circulating GH profile of a subgroup of our cohort in more detail, 35 men and 36 women were admitted to hospital for overnight rest, and an indwelling iv cannula was inserted. At 0730 h, a venous blood sample was drawn for measurement of serum GH concentration, and samples were drawn every 20 min thereafter for 24 h. Standard meals were taken at 0800, 1230, and 1800 h, and a normal daily routine was encouraged within the confines of the hospital. At 0600 h, an additional blood sample was drawn for the measurement of IGF-I, IGF-binding protein-1 and -3, and GH-binding protein. An overnight urine collection was also made. Women taking hormone replacement therapy and those with diabetes mellitus were excluded.

GH was measured using a chemiluminescence technique (Nichols Institute, San Juan Capistrano, CA). The within-assay coefficients of variation (CVs) were 5.5%, 6.8%, and 10.5% at serum concentrations of 0.4, 10.0, and 18.6 mU/liter, respectively, and the between-assay coefficients of variation were 12.1%, 12.3%, and 9.0% at serum concentrations of 3.3, 6.3, and 18.0 mU/liter, respectively. The sensitivity of the assay was 0.05 mU/liter. Standards in the assay were calibrated against the International Reference Preparation (80/505).

GH profiles were analyzed to derive the following values. The circulatory profile of GH was characterized by estimating the concentration at or below which 5%, 50%, or 95% of the time is spent from the total of 72 values measured in each subject (11).

Serum IGF-I was measured using an in-house polyclonal RIA with acid-alcohol extraction. The sensitivity of the assay was 0.07 U/ml. The within-assay CVs were 11.3%, 6.5%, and 4.7% at serum concentrations of 0.23, 1.23, and 3.53 U/ml, respectively; the between-assay CVs were 10.5%, 12.1%, and 5.1% at concentrations of 0.38, 0.99, and 3.54 U/ml, respectively. IGF-binding protein-3 was measured using an RIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX). The sensitivity of the assay was 0.9 ng/ml. The within-assay CVs were 8.1% and 5.4% at serum concentrations of 2200 and 7800 ng/ml, respectively, whereas the between-assay CVs were 9.8% and 4.8% at serum concentrations of 2200 and 8500 ng/ml, respectively. Estradiol was measured by a competitive RIA using ^{125}I tracer (Diagnostic Products Corp., Los Angeles, CA). The between-assay CVs were 10%, 7.9%, and 7.7% at 230, 1003, and 1456 pmol/liter, respectively.

Serum osteocalcin was measured in a fasting blood sample using an RIA with antiserum raised to human osteocalcin and human osteocalcin as the standard and tracer (Nichols Institute). The intraassay CV was 3%; the interassay CV was 5% at 4.8 mg/liter, and the detection limit was 0.3 mg/liter. A 2-h morning urine sample was also obtained, after overnight fasting. Type I collagen cross-linked N-telopeptide (NTX/Cr) was measured using an ELISA (Ostex, Inc., Seattle, WA). The intraassay CV was 3.8%, and the interassay coefficient of variation was 6.2%. Creatinine was quantified in urine samples by the Jaffe procedure, and cross-link excretion was expressed as a ratio to urinary creatinine concentration.

The normality of variables was assessed, and variables were transformed as required. All statistical analyses were carried out using STATA (release 7.0, Stata Corporation, College Station, TX). ANOVA and linear regression were used to examine the associations between genotype and both BMD and bone loss rate at the lumbar spine and proximal femur. Multiple linear regression was performed to adjust for the effects of adult lifestyle variables that influence bone density and bone loss. Some genotypes were rare; hence, we performed some analyses dichotomizing for the presence/absence of an allele. GH haplotypes were inferred using the PHASE package (18). Bone phenotypes were related to phased GH gene haplotypes using multiple linear regression based on haplotype count variables (coded using STATA). The magnitude (D′) and the significance of pairwise linkage disequilibrium (LD) between *GH1* polymorphisms were computed by the 2LD program (36). D' is defined as D/D_{max}, where $D = f(AB) - pu$, with $f(AB)$ being the frequency of the AB haplotype, and p and u being the allele frequencies at the two loci. D_{max} is minimum $[p(1-u), (1-p)u]$, when $D > 0$, or minimum $[pu, (1-p)(1-u)]$, when $D < 0$. D' ranges from −1 to 1, −1, and 1, indicating complete LD, and 0, indicating complete lack of LD. The significance of LD was determined from χ^2, with χ^2 being $N D^2/p(1-p)u(1-u)$, where N is the sample size.

All subjects gave informed written consent, and permission to conduct the study was granted by the East and North Hertfordshire ethical committees.

Results

The characteristics of the study population at baseline are shown in Table 1. The mean age of the men studied was 66.1 yr, and that of the women was 65.5 yr. Their mean birth weights were 3.6 and 3.5 kg, respectively, and calcium intake was higher among men (715 mg daily) than among women (654 mg daily). Eighty-two percent of the men and 43.9% of the women had ever smoked, and the median weekly alcohol consumption was 6.0 U for men and 0.5 U for women.

The frequency distributions of the genotypes are as follows and did not differ by sex: GHV1, 11–99 (31.9%), 12–138 (44.4%), and 22–74 (23.8%); and GHV4, 11–108 (36.3%), 12–134 (45.2%), and 22–53 (18.5%), which were tested for and found to be in Hardy-Weinberg equilibrium. GHV1 and GHV4 were in strong LD ($D' = 0.662$; $P < 10^{-20}$).

Table 2 shows the baseline bone density and annualized bone loss rate among men and women in the study according to allelic variation in the GHV1 and GHV4 SNPs. Observations were similar among men and women, and data are presented for both sexes combined after adjusting for age, gender, calcium intake, body weight, social class, smoking status, alcohol consumption, physical activity, and osteoarthritis (Kellgren and Lawrence score) (16). There were no statistically significant associations between either genetic marker with baseline bone density at the lumbar spine or proximal femur. There were, however, significant associations of each marker with the rate of bone loss at the lumbar spine and proximal femur. The 2 allele at the GHV1 locus was associated with significantly greater loss rates at the spine ($P = 0.03$) and hip ($P = 0.04$), whereas the 2 allele at the GHV4 locus was associated with a significantly greater loss rate at the spine ($P = 0.008$).

TABLE 1. Characteristics at baseline among 205 men and 132 women, aged 61–73 yr

	Men	Women
Age (yr)	66.1 (3.2)	65.5 (2.6)
Body mass index (kg/m^2)	26.9 (3.4)	26.7 (4.2)
Calcium intake (mg/d)	715 (241)	654 (215)
Outdoor walking (min/d)	98.2 (92.6)	84.4 (56.2)
Baseline BMD (g/cm^2)		
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 (0.1)	0.8 (0.1)
Annualized change in bone density (%/yr)[a]		
Lumbar spine	0.4 (1.3)	−0.3 (1.2)
Femoral neck	−0.3 (1.5)	−1.1 (1.4)
Total proximal femur	−0.1 (1.4)	−1.3 (1.2)

Values are means (±SD).

[a] A negative figure implies bone loss over the follow-up period.

TABLE 2. Bone density and annualized change over 4 yr, according to GH genotype in 205 Hertfordshire men and 132 Hertfordshire women, aged 61–73 yr

	GHV1 genotype			GHV4 genotype		
	No.	Mean	SD	No.	Mean	SD
Lumbar spine BMD						
11	99	1.02	(0.19)	108	0.99	0.20
12	138	1.01	(0.18)	134	1.04	0.20
22	74	1.01	(0.20)	53	0.99	0.16
	$P = 0.71$ on 2df			$P = 0.15$ on 2df		
Lumbar spine BMD change						
11	91	0.24	(1.32)	95	0.21	1.42
12	121	0.19	(1.32)	120	0.28	1.17
22	67	−0.24	(1.33)	47	−0.42	1.25
	$P = 0.03$ for trend			$P = 0.008$ on 2df		
Total femoral BMD						
11	98	0.94	(0.14)	107	0.91	0.14
12	138	0.91	(0.15)	134	0.92	0.17
22	74	0.91	(0.16)	53	0.92	0.13
	$P = 0.06$ on 2df			$P = 0.93$ on 2df		
Total femoral BMD change						
11	90	−0.50	(1.21)	94	−0.54	1.32
12	121	−0.59	(1.60)	120	−0.66	1.14
22	67	−0.78	(1.30)	47	−0.91	1.28
	$P = 0.04$ for trend			$P = 0.57$ on 2df		

Descriptive statistics are for men and women combined, and regression models/ANOVAs were carried out for men and women combined. A test based on 2df or a test for trend was performed according to whether the relationship observed appeared allele based or genotype based. *P* values are from analyses adjusted for gender, age, calcium intake, weight, social class, smoking status, alcohol intake, activity, and osteoarthritis score.

Serum osteocalcin was higher in men carrying the 1 allele of GHV1 in a dose-dependent effect ($P = 0.04$, adjusted for age, calcium intake, weight, social class, cigarette and alcohol consumption, and physical activity), although no relationships were seen between genotype and NTX/Cr. By contrast, women carrying the GHV1 2 allele displayed higher NTX/Cr levels with a dose-dependent effect ($P = 0.03$ adjusted for age, calcium intake, weight, social class, cigarette and alcohol consumption, and physical activity). There were no significant associations between these biochemical markers of bone turnover and GHV4 in either sex.

Haplotype analysis revealed a total of seven commonly occurring haplotypes for the two SNPs of interest (GHV1 and GHV4): 1 2 1; 2 2 1; 2 2 2; 1 2 2; 2 1 2; 2 1 1; and 1 1 1. Haplotype 1 (1 2 1) was set as the reference. Significant associations were observed between haplotypes containing the 2 allele at GHV1 and/or GHV4, and reduced bone density (as well as accelerated bone loss) at the lumbar spine, replicating our previous findings. Results at the proximal femur were again weaker and were not statistically significant. Hence, our findings suggest that the genotypes GHV1 and GHV4, or polymorphic genes in LD, are acting as allelically associated markers within the same haplotype block and are associated with accelerated bone loss rate in the seventh decade.

Thirty-five men and 36 women also underwent detailed characterization of their circulating GH profiles, as previously described. We have previously observed differing profiles of GH concentration according to gender in this cohort (19) and hence analyzed relationships with genotype in each gender separately (Table 3). Among men, the skeletally unfavorable 2 allele at the GHV1 locus was associated with lower trough ($P = 0.03$), median ($P = 0.06$), and peak ($P = 0.07$) 24-h GH concentrations, as displayed graphically in Fig. 1. In women, although analyses were also limited by the small sample size, there did not appear to be any association between GH genotype and circulating GH concentration. Similarly, there were no relationships observed between GHV4 genotype and circulating GH concentration. The sexual dimorphism observed with regard to the relationships between GHV1 genotype and GH concentration was mirrored in relationships between bone loss rate and GH concentration; relationships were significant in men, but not in women, at the total femoral region with trough ($r = 0.39$; $P = 0.02$) and median ($r = 0.43$; $P = 0.01$) concentrations. Similar relationships were observed at the femoral neck. We found no evidence of gender-GHV1 allelic interaction in the prediction of BMD or bone loss rate at any site.

Associations were also seen between the different alleles and weight in infancy, although these tended not to attain statistical significance. Hence, the GHV1 2 allele tended to be associated with lower weight at 1 yr in men. Again, there were no trends suggestive of an association between early weight and GH genotype in women.

Finally, we investigated the possible interaction between genotype and early environment as predictors of bone mass and bone loss (Table 4). These analyses were performed in view of the associations previously demonstrated between another genotype associated with bone mass (the *VDR* gene) and early life (12). The table shows the annualized change in lumbar spine bone density according to weight at 1 yr and GHV1 genotype. Individuals in the lowest third of the distribution of weight at 1 yr showed a strong relationship

TABLE 3. Twenty-four-hour GH profile according to GH genotype in 35 Hertfordshire men, aged 61–73 yr

24-h GH conc.	GHV1 genotype		
	No.	Mean	SD
Trough			
11	13	0.10	1.6
12	16	0.08	1.5
22	5	0.05	2.4
	$P = 0.03$ for trend		
Median			
11	13	0.31	1.9
12	16	0.23	1.7
22	5	0.17	1.9
	$P = 0.06$ for trend		
Peak			
11	13	4.23	1.9
12	16	3.61	2.2
22	5	2.50	2.0
	$P = 0.07$ for trend		

Geometric means and SD values are presented for all 24-h GH concentration variables. *P* values are from analyses adjusted for age, calcium intake, weight, social class, smoking status, alcohol intake, and activity.

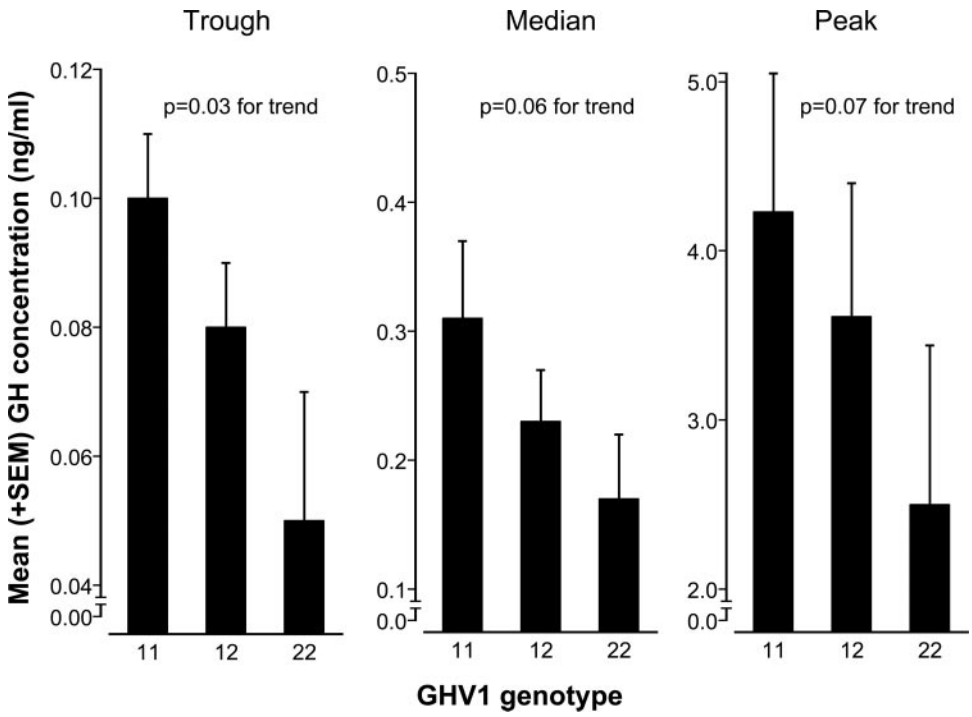

FIG. 1. SNPs in the *GH1* gene promoter and 24-h circulating GH profile in 35 Hertfordshire men.

TABLE 4. Annualized change [mean (SD)] in lumbar spine bone density in Hertfordshire men and women according to GHV1 genotype and weight at 1 yr

GHV1	Weight at 1 yr (tertile)		
	1st	2nd	3rd
11	0.41 (1.21)	0.38 (1.47)	−0.05 (1.25)
	28	*31*	*32*
12	−0.02 (1.47)	0.02 (1.26)	0.54 (1.17)
	41	*37*	*43*
22	−0.53 (1.48)	0.05 (0.90)	−0.17 (1.47)
	27	*21*	*19*

The number of subjects is in *italics*. Regression models were carried out for men and women combined. Test for interaction: $P = 0.04$ on 4df adjusted for gender, age, calcium intake, weight, social class, smoking status, alcohol intake, activity, and osteoarthritis score.

between GHV1 genotype and bone loss rate (difference in mean bone loss rate, 0.94% between 11 and 22 genotypes), whereas these relationships were not apparent for subjects in the intermediate or higher thirds of the infant weight distribution. Test for interaction was significant ($P = 0.04$, after adjusting for potential confounding variables).

Discussion

We have demonstrated associations between SNPs in the *GH1* gene and bone loss rate in a Hertfordshire population of healthy elderly men and women. These genetic variants were also associated with alterations in the circulating GH profile in a subgroup of the original cohort. To our knowledge, this is the first report relating *GH1* genotype to osteoporosis risk.

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, UK, and still live there. They have, however, previously been shown to have anthropometric and lifestyle characteristics similar to those of the general population (20). Not all subjects who participated in the baseline study (224 men and 186 women) were seen at follow-up 4 yr later, but we found no difference in baseline body build, lifestyle, and BMD between those who did and did not participate in the whole study (15). The lesser relationships observed at the lumbar spine may reflect arthritic changes; we attempted to adjust for this by including an osteoarthritis score according to the methods of Kellgren and Lawrence (16) in our regression models, although another approach may have been to examine relationships with the L1 vertebra only, because this vertebra is often uninvolved in osteoarthritic change. The study performed was small in comparison with many genetic studies, and the allelic frequency of some genotypes limited power for some analyses. Hence, although this study had 77% power to detect a significant difference in lumbar spine BMD change between the two homozygotes, 11 and 22, of the GHV4 genotype, this figure fell to 36% power when looking for a significant difference in femoral neck BMD change between the two homozygotes, 11 and 22, of the GHV4 genotype. Hence, our findings are all the more striking and require validation in a larger study powered to examine genetic effects on bone mass.

GH stimulates linear growth in childhood and bone remodeling throughout life. It stimulates chondrocytes in the growth plate to secrete IGF-I, which, in turn, signals the chondrocytes to differentiate, leading to cartilage formation and linear growth. In addition, GH probably has effects independent of IGF-I. Although the role of GH in the risk of osteoporosis is unclear, GH deficiency is associated with low adult BMD (19–23), although this is often difficult to correct with GH treatment (24–27). Case-control studies also show lower circulating IGF-I levels in patients with osteoporosis

than in normal controls (28). The pulsatile nature of GH secretion is controlled by two hypothalamic hormones with opposing effects: GHRH and somatostatin or GH release-inhibiting hormone (29). The biological function of this pulsatile pattern is unknown, but the amplitude of GH peaks correlate with height during childhood (30, 31), suggesting that the GH concentration may be an important determinant of skeletal growth.

The *GH1* gene is located on chromosome 17q23; although little is known about the role of polymorphisms in the gene, case reports of mutations in the GH receptor have been described (32–34). Other work in animals has focused on the effects of GH oversecretion on bone modeling and remodeling in transgenic mice (35); this reveals that continual systemic stimulation by GH results in an initial increase in bone mass, but that bone quality is unfavorably affected. The proximal promoter region of the *GH1* gene is highly polymorphic, containing at least 15 SNPs manifesting in 40 different haplotypes. Recently reported functional studies using an *in vitro* assay of reporter gene expression have suggested that 12 of these haplotypes are associated with significantly reduced expression, whereas 10 are associated with increased levels (14). Although measurements of skeletal status were not available in that study, a relationship was reported between an individual's *GH1* promoter haplotype combination and adult height. The results of both studies suggest that this locus warrants closer scrutiny in explaining the complex interindividual differences in skeletal size, bone density, and bone loss.

Separate studies have related GHV1 to GH transcription *in vitro* (14) and GHV4 to growth retardation (13). We have examined both markers in this study; their strong LD (>60%; $P < 10^{-20}$) explains the similarity of their associations with bone loss. It also permits comparison between our study of osteoporosis phenotypes and GH secretion and previous reports of GH gene function *in vitro* and GH1 sequence polymorphism in growth retardation. Additional analyses in this cohort demonstrate strong LD not only across the GH gene, but also extending to the adjacent placental *GH2* and lactogen gene cluster, and moderate LD as far as the angiotensin-converting enzyme gene locus situated an estimated 1 megabase away (34). The most obvious hypothetical chain of causality is that GH gene diversity influences GH expression and circulating levels (quantitative on allele), with consequent unfavorable effects on skeletal health, most notably in homozygotes for low secretion. However, strong LD across not only *GH1*, but the entire *GH1-CSH1-GH2-CSH2* gene cluster, means that identification and proof of a causal site(s) (as opposed to proxy markers and risk haplotype(s)) may be difficult.

We have previously reported data suggesting that the intrauterine environment, using birth weight as a marker, may modulate the relationship between the *VDR* gene and adult bone size and volumetric density (12). This study provides evidence of a significant interaction between the early environment and a second genotypic locus, such that individuals who carry the unfavorable genotype, who also grew poorly in fetal life and infancy, are susceptible to accelerated bone loss in the seventh decade. Additional studies to identify the mechanisms for this association are required.

In conclusion, our study implicates, for the first time, common diversity in the *GH1* region in predisposing to osteoporosis via effects on the level of GH expression. The interaction with infant weight suggests that early environmental influences may modulate the effect of *GH1* genotype on bone loss some 7 decades later. Larger replication studies, with expansion of the range of phenotypes examined and comprehensive studies of common diversity in the *GH1* region, need to be established

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References

1. **Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J** 1989 Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 320:554–558
2. **Kelly PJ, Eisman JA, Sambrook PN** 1990 Interaction of genetic and environmental influences on peak bone density. Osteoporos Int 1:56–60
3. **Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S** 1987 Genetic determinants of bone mass in adults: a twin study. J Clin Invest 80:706–710
4. **Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CC** 1991 Genetic determinants of bone mass in adult women: a re-evaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res 6:561–567
5. **Gueguen R, Jouanny P, Guillermin F, Kuntz C, Powell J, Siest G** 1995 Segregation analysis and variable components analysis of bone mineral density in healthy families. J Bone Miner Res 12:2017–2022
6. **Ralston S** 1998 Do genetic markers aid in risk assessment? Osteoporos Int 8(Suppl 1):S37–S42
7. **Albagha OM, McGuigan FE, Reid DM, Ralston SH** 2001 Estrogen receptor α gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. J Bone Miner Res 16:128–134
8. **Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L, Barker D** 1995 Childhood growth, physical activity and peak bone mass in women. J Bone Miner Res 10:940–947
9. **Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D** 1997 Growth in infancy and bone mass in later life. Ann Rheum Dis 56:17–21
10. **Jones G, Riley M, Dwyer T** 1999 Maternal smoking during pregnancy, growth, and bone mass in prepubertal children. J Bone Miner Res 14:146–151
11. **Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D, Cooper C** 1998 Programming of growth hormone secretion and bone mineral density in elderly men: an hypothesis. J Clin Endocrinol Metab 83:135–139
12. **Dennison EM, Arden NK, Keen RW, Syddall H, Day INM, Spector TD, Cooper C** 2001 Birthweight, vitamin D receptor genotype and the programming of osteoporosis. Paediatr Perinat Epidemiol 15:211–219
13. **Hasegawa Y, Fujii K, Yamada M, Igarashi Y, Tachibana K, Tanaka T, Onigata K, Nishi Y, Kato S, Hasegawa T** 2000 Identification of novel human GH-1 gene polymorphisms that are associated with growth hormone secretion and height. J Clin Endocrinol Metab 85:1290–1295
14. **Horan M, Millar DS, Hedderich J, Lewis G, Newsway V, Mo N, Fryklund L, Procter AM, Krawczak M, Cooper DN** 2003 Human growth hormone 1 (GH1) gene expression: complex haplotype-dependent influence of polymorphic variation in the proximal promoter and locus control region. Hum Mutat 21:408–423

15. **Dennison E, Eastell R, Fall CHD, Kellingray S, Wood PJ, Cooper C** 1999 Determinants of bone loss in elderly men and women: a prospective population-based study. Osteoporos Int 10:384–391
16. **Kellgren JH, Lawrence JS** 1963 Atlas of standard radiographs: the epidemiology of chronic rheumatism, vol 2. Oxford: Blackwell
17. **Gaunt TR, Hinks LJ, Rassoulian H, Day IN** 2003 Manual 768 or 384 well microplate gel 'dry' electrophoresis for PCR checking and SNP genotyping. Nucleic Acids Res 31:e48
18. **Stephens M, Smith NJ, Donnelly P** 2001 A new statistical method for haplotype reconstruction from population data. Am J Horm Genet 68:978–989
19. **Charmandari E, Pincus SM, Matthews DR, Dennison E, Fall CHD, Hindmarsh PC** 2001 Joint growth hormone and cortisol spontaneous secretion is more asynchronous in older females than in their male counterparts. J Clin Endocrinol Metab 86:3393–3399
20. **Egger P, Duggleby S, Hobbs R, Fall C, Cooper C** 1996 Cigarette smoking and bone mineral density in the elderly. J Epidemiol Community Health 50:47–50
21. **DeBoer H, Blok GJ, VanLingen A, Teule GJJ, Lips P, Van derVeen EA** 1994 Consequences of childhood-onset growth hormone deficiency for adult bone mass. J Bone Miner Res 9:1319–1326
22. **Kaufman J-M, Taelman P, Vermeulen A, Van de Weghe M** 1992 Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. J Clin Endocrinol Metab 74:118–123
23. **Hyer SL, Rodin DA, Tobias JH, Leiper A, Nussey SS** 1992 Growth hormone deficiency during puberty reduces adult bone mineral density. Arch Dis Child 67:1472–1474
24. **Johansson A, Lindh E, Ljunghall S** 1993 IGFs: function and clinical importance: growth hormone, insulin-like growth factor I, and bone: a clinical review. J Intern Med 234:553–560
25. **Kassem M, Brixen K, Blum WF, Mosekilde L, Eriksen EF** 1994 Normal osteoclastic and osteoblastic responses to exogenous growth hormone in patients with postmenopausal spinal osteoporosis. J Bone Miner Res 9:1365–1370
26. **Clemmesen B, Overgaard K, Riis B, Christiansen C** 1993 Human GH and GHRH: a double masked, placebo controlled study of their effects on bone metabolism in elderly women. Osteoporos Int 3:330–336
27. **Marcus R, Butterfield G, Holloway L, Gilliland L, Baylink DJ, Huntz RL, Sherman BM** 1990 Effects of short-term administration of recombinant human GH to elderly people. J Clin Endocrinol Metab 70:519–527
28. **Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW, Mattson DE** 1990 Effect of human growth hormone in men over age 60. N Engl J Med 323:52–54
29. **Cronin MJ, Thorner MO** 1989 Basic studies with GH releasing factor. In: De Groot LJ, ed. Endocrinology. Philadelphia: Saunders; vol 1:183–191
30. **Hindmarsh P, Smith PJ, Brook CGD, Matthews DR** 1987 The relationship between height velocity and growth hormone secretion in short prepubertal children. Clin Endocrinol (Oxf) 27:581–591
31. **Albertsson-Wikland K, Rosberg S** 1988 Analyses of 24-hour growth hormone profiles in children: relation to growth. J Clin Endocrinol Metab 67:493–500
32. **Walker JL, Crock PA, Behncken SN, Rowlinson SW, Nicholson LM, Boulton TJ, Waters MJ** 1998 A novel mutation affecting the interdomain link region of the growth hormone receptor in a Vietnamese girl, and response to long-term treatment with recombinant human insulin-like growth factor-I and luteinizing hormone releasing hormone analogue. J Clin Endocrinol Metab 83:2554–2561
33. **Sjoberg M, Salazar T, Espinosa C, Dagnino A, Avila A, Eggers M, Cassorla F, Carvallo P, Mericq MV** 2001 Study of GH sensitivity in Chilean patients with idiopathic short stature. J Clin Endocrinol Metab 86:4375–4381
34. **Millar DS, Lewis MD, Horan M, Newsway V, Easter TE, Gregory JV, Fryklund L, Norin M, Crowne EC, Davies SJ, Edwards P, Kirk J, Waldron K, Smith PJ, Phillips III JA, Scanlon MF, Krawczak M, Cooper DN, Procter AM** 2003 Novel mutations of the growth hormone 1 (GH1) gene disclosed by modulation of the clinical selection criteria for individuals with short stature. Hum Mutat 21:424–440
35. **Steinke B, Patwardhan AG, Havey RM, King D** 1999 Human growth hormone transgene expression increases the biomechanical structural properties of mouse vertebrae. Spine 24:1–4
36. **Zapata C, Carollo C, Rodriguez S** 2001 Sampling variance and distribution of the D′ measure of overall gametic disequilibrium between multiallelic loci. Ann Hum Genet 65:396–406

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