

Pharmacogenetic risk factors for altered bone mineral density and body composition in pediatric acute lymphoblastic leukemia

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ABSTRACT

Background

This study investigates pharmacogenetic risk factors for bone mineral (apparent) density (BM(A)D) and body composition in pediatric acute lymphoblastic leukemia

Design and Methods

We determined the influence of SNPs in 4 genes (*vitamin-D receptor (VDR: BsmI/ApaI/TaqI and Cdx-2/GATA)*, *collagen type I alpha 1 (SpI)*, *estrogen receptor 1 (ESR1: PvuII/XbaI)*, *glucocorticoid receptor (BclI)*) on body composition, BM(A)D and fracture risk during dexamethasone-based pediatric acute lymphoblastic leukemia treatment. Body composition and BMD were measured repeatedly during and after treatment using dual energy X-ray absorptiometry.

Results

Non-carriers of *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 revealed a significant larger fat gain than carriers ($\Delta\%$ fat: non-carriers: +1.76SDS, carriers: +0.77SDS, $P < 0.001$). At diagnosis and during therapy, lumbar spine BMD was significantly higher in non-carriers of *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 than in carriers. The other SNPs did not influence BMD or fracture risk during/after treatment. The year after treatment completion, lean body mass increased in non-carriers of *ESR1* (*PvuII/XbaI*) haplotype 3 and decreased in carriers (Δ lean body mass: non-carriers: +0.28SDS, carriers: -0.55SDS, $P < 0.01$).

Conclusions

Only *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 was identified as protective factor against excessive fat gain and as a risk factor for lower lumbar spine BMD during treatment. Carrying *ESR1* (*PvuII/XbaI*) haplotype 3 negatively influenced recovery of lean body mass after pediatric acute lymphoblastic leukemia treatment.

Key words: *Cdx-2/GATA*, pharmacogenetic risk factors, acute lymphoblastic leukemia.

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Introduction

As the cure rate of pediatric acute lymphoblastic leukemia (ALL) is high,¹ research on treatment-related morbidity, like disturbance of body composition and bone mineral density (BMD), is required.²⁻⁴ Leukemia and its treatment, especially corticosteroids⁵ and methotrexate,⁶ may lead to reduced BMD. However, uniformly treated children show a large variation in disturbance of body composition, BMD reduction and fractures, suggesting a role for pharmacogenetics in the pathogenesis of these problems.⁴

Several single nucleotide polymorphisms (SNPs) have been shown to influence BMD in adults, especially those of the vitamin-D receptor gene (*VDR*).⁷⁻¹² The extent of the influence of *VDR* SNPs on BMD may be dependent on age and menopausal state.¹³ In healthy children, only a few studies on the influence of *VDR* 3'-end SNPs (*BsmI*, *ApaI*, *TaqI*) on BMD have been reported, with conflicting results.¹⁴⁻¹⁷ Effects of *VDR* 5'-promoter SNPs (*Cdx-2*, *GATA*) on BMD have not been investigated in healthy children. With regards to body composition (muscle strength and fat mass), it has been demonstrated that the *VDR BsmI* SNP determines body composition in premenopausal women.¹⁸

Another polymorphism frequently reported to be associated with a low BMD for chronological age is the G→T substitution in the *Sp1* binding site of the collagen type I alpha 1 gene (*COL1A1*). This can result in increased expression of collagen type I alpha 1 in the bone matrix.^{7, 19-21} Studies regarding the relationship between BMD and carrying *COL1A1* risk alleles in healthy children show conflicting results.²²⁻²⁵

Haplotypes of the 5'-end of the estrogen receptor alpha gene (*ESR1*) in which the risk alleles of the *PvuII* and *XbaII* SNPs are combined are associated with decreased BMD and fractures in post-menopausal women.²⁶⁻²⁸ Only a few studies in healthy children have been carried out, showing conflicting results concerning the influence of *ESR1* risk alleles on BMD.^{25, 29} On the other hand, the *PvuII* and *XbaII* SNPs are not related to body composition in healthy children.³⁰

In healthy adults, it has been suggested that polymorphisms in the glucocorticoid receptor gene (*GR*), like *BclI* and *N363S*, modulate corticosteroid sensitivity.^{31, 32} This in turn could result in reduced BMD^{32, 33} and disturbed body composition.^{31, 34} Since corticosteroids are considered to cause altered body composition and reduced BMD, we hypothesize that *GR* SNPs may influence variation in body composition and BMD in pediatric ALL.

To our knowledge, this is the first study investigating the influence of genetic variation of the *VDR*, *COL1A1*, *ESR1* and *GR* on BMD, body composition and fracture risk in pediatric ALL patients during and after therapy. The aim of this study is to identify patients at risk for a low BMD for chronological age and a disturbed body composition, in order to develop early preventative interventions.

Design and Methods

Patients

In this prospective study, children with newly diagnosed ALL were treated according to the dexamethasone-based protocol of the Dutch Childhood Oncology Group (DCOG-ALL9).³⁵ High-

risk criteria were white blood cell count of $50 \times 10^9/L$ or over, T-cell immunophenotype, mediastinal mass, central nervous system involvement, testes infiltration, t(9;22) and 11q23/*MLL* gene rearrangements. The treatment schedules included dexamethasone given in repetitive pulses (cumulative dose: 1,244 mg/m² (high-risk) and 1,370 mg/m² (non-high risk)). Total cumulative dose of methotrexate was 13,650 mg/m² in the high-risk protocol and 8,100 mg/m² in the non-high risk protocol. No patient received central nervous system irradiation.

To determine a potential selection bias, we compared patient characteristics of participants of the current study with those of the total Rotterdam DCOG-ALL9-treated cohort. The Medical Ethical Committee approved the study. Written informed consent according to the Helsinki agreement was obtained from all parents and patients aged 12 years and older.

Polymorphisms

After reaching complete remission, germ-line genomic DNA was extracted from a minimum of 5.0×10^6 peripheral blood mononuclear cells using TRIzol reagent (Gibco BRL, Life Technologies) according to the manufacturer's protocol. The DNA was quantified using spectrophotometry. Figure 1 shows positions of the SNPs which were detected by real-time PCR and hybridization probes (Taqman).

We determined three SNPs at the 3'-end of the *VDR* gene (*BsmI* (E8-G+284A, rs1544410), *ApaI* (E9-T-48G, rs739837), and *TaqI* (E9-T32C, rs731236)).¹² Haplotypes were named as previously described.^{9, 12} In our patients, haplotype 1 (baT), haplotype 2 (BaT), haplotype 3 (bAT) and haplotype 4 (BAT) occurred, which combined to eight genotypes encoded 1/1, 1/2, 1/3, 1/4, 2/2, 2/3, 2/4 and 3/3 (3/4 and 4/4 were not observed).

Two other *VDR* 5'-promoter region SNPs were studied; the G→A substitution in the *Cdx-2* binding site (1e-G-1739A, rs11568820) and an A→G substitution in the *GATA* binding site (1a-A-1012G, rs4516035)[8, 9, 36]. Both 5'-promoter polymorphisms were combined to haplotype 1 (GA), haplotype 2 (GG) and haplotype 3 (AG), combining to six genotypes encoded as 1/1, 1/2, 1/3, 2/2, 2/3 and 3/3.

The *Sp1* polymorphism is a G→T substitution affecting a binding site of the *Sp1* transcription factor in the first intron of *COL1A1* (int1-G+1245T, rs1800012).³⁷ The polymorphism results in three genotypes GG, GT and TT.

We genotyped two polymorphisms in the first intron of *ESR1*: *PvuII* (int1-T-397C, rs2234693) and *XbaI* (int1-A-351G, rs9340799).²⁸ Three haplotype alleles were encoded as haplotype 1 (px), haplotype 2 (PX), and haplotype 3 (pX), combining to six genotypes 1/1, 1/2, 1/3, 2/2, 2/3 and 3/3.

We determined two SNPs of the *GR*: the *BclI* (int2-C-646G, rs not available) which combined to the genotypes CC, CG and GG and the *N363S* (e2-A1218G, rs6195) combining to the genotypes AA, AG and GG.³⁸

End points

Anthropometry data were measured in all patients. Height was measured with a Harpenden stadiometer and weight with standard clinical scales. The body mass index (BMI) was calculated as weight/height². Height and BMI of the patients were compared with reference values of healthy controls matched for age and sex and expressed as standard deviation scores (SDS).^{39, 40}

In patients aged four years and older, dual energy X-ray absorptiometry (DXA, Lunar DPX-L) provided estimates of lean body mass (LBM), percentage fat of the total body (%fat_{TB}), BMD of the total body (BMD_{TB}) and BMD of the lumbar spine (BMD_{L5}). To correct for bone size, we calculated bone mineral apparent density (BMAD) of the lumbar spine with the model

$BMD_{LS} = BMD_{Sx} \times (4 / (\pi \times \text{width}))$. 'Width' is the mean width of the second to the fourth lumbar vertebrae. This model was validated by *in vivo* volumetric data obtained from magnetic resonance imaging.⁴¹ All DXA results were expressed as age-matched and sex-matched SDS.⁴² Special pediatric software was used for children who weighed less than 30 kg.

Symptomatic fractures, confirmed by radiography, were registered. Fracture incidence rates of the various allelic variants were calculated. In addition, incidence-rate ratios for non-carrier versus carriers were calculated.

Habitual physical activity measured in minutes/week included physical education classes, organized sports, recreational activities, habitual walking/ cycling.⁴³ Calcium intake was determined by a detailed food-frequency questionnaire of dairy products.⁴⁴ Serum calcium, 1,25-dihydroxy-vitamin D and PTH were assessed. Since over time, PTH concentrations were measured on three different immunoanalyzers, concentrations of PTH were expressed as the number of standard deviations above the upper limit of the reference range of the immunoassay used.⁴⁵

Measurements were performed at diagnosis, after 32 weeks, one year, two years (completion of therapy) and three years (one year after completion of therapy). Differences between non-carriers and carriers in change of end points during the two-year treatment period (Δ_1) and during the first year after completion of chemotherapy (Δ_2) were investigated. To compare positions of the curves of the different carrier groups, areas under the curves were calculated.

Statistical analysis

SNPs were tested for deviation from the Hardy Weinberg Equilibrium (HWE) by comparing the observed and expected genotype frequencies using a χ^2 test. We calculated areas under the curves using the trapezium rule. A Mann-Whitney U-test/ χ^2 -test was used to compare baseline patient characteristics and areas under the curves for the different carrier groups.

Anthropometry, body composition and BMD at diagnosis were compared with normal reference values using a one-sample t-test. Fracture incidence-rate ratios were tested using Poisson statistics. These statistical analyses were performed with SPSS 15.0 (SPSS Inc. Chicago, IL, USA). Differences between the carrier groups in changes of end points (Δ_1 and Δ_2) were analyzed using repeated measurements analysis (SAS PROC MIXED, SAS Institute Inc., North Carolina, USA), with an unstructured repeated covariance type. We pooled heterozygous and homozygous carriers under a dominant inheritance model. In view of the multiple comparisons, *P* values of ≤ 0.01 were considered to be significant. All analyses were carried out according to the intention-to-treat principle; for children who did not complete the study, data prior to elimination were included.

Results

Patients

Sixty-nine patients (39 males) were included, with a mean age of 7.4 (range 1.6-16.8) years. Twenty patients were treated with the high-risk protocol and the remaining children received non-high risk treatment. Age, gender and risk-group stratification of the included patients was similar to that of the total DCOG-ALL9-treated cohort, which indicated that the sample constituted a representative selection of the Rotterdam cohort.

Genotype distribution

The distribution of the genotypes of *VDR* 3'-end (*BsmI*/*Apal*/*TaqI*) and 5'-end (*Cdx-2*/*GATA*), *COLIA1* (*Sp1*), *ESR1* (*PvuII*/*XbaI*), and *GR* (*BclI*) were in HWE (Table 1). No homozygous carriers and only 3 heterozygous carriers of the *GR* (N363S) polymorphism were determined (*data not shown*).

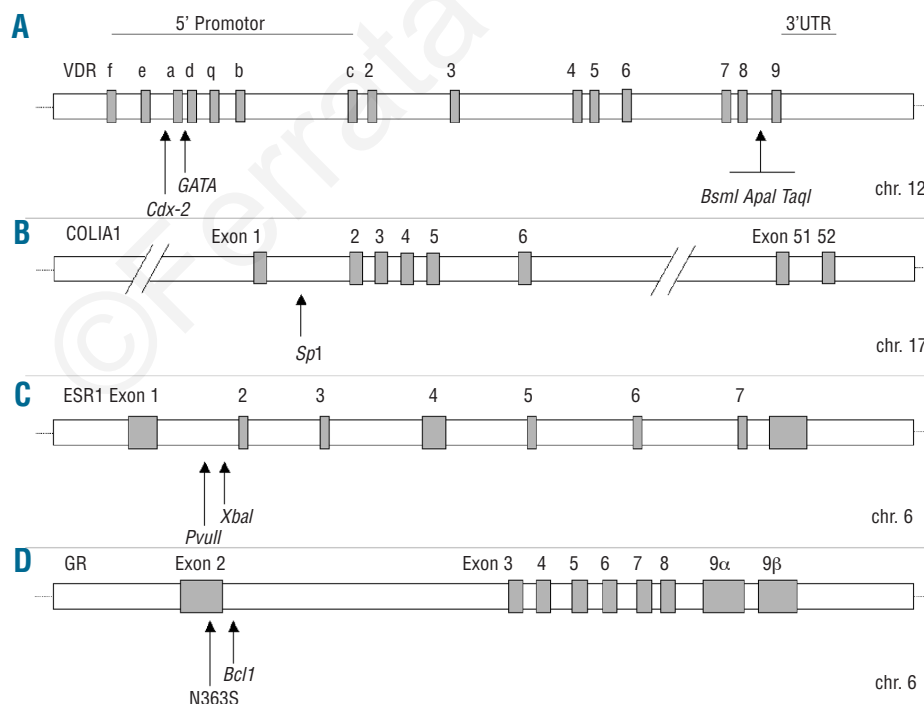


Figure 1. Genomic structure and positions of the single nucleotide polymorphisms investigated in current study. (A) The vitamin-D receptor gene (*VDR*). (B) The collagen type I $\alpha 1$ gene (*COLIA1*). (C) The estrogen receptor α gene (*ESR1*). (D) The glucocorticoid receptor gene (*GR*). UTR: untranslated region; chr.: chromosome.

Baseline data

At diagnosis, there was no difference in %fat_{TB} of our sample compared with that of healthy peers. The patients showed a lower BMI and LBM at diagnosis than healthy peers (BMI=-0.51SDS, $P<0.01$ and LBM=-0.67SDS, $P<0.001$). There was no difference in baseline BMD_{TB} of our ALL patients compared with healthy peers, whereas BMD_{LS} of the patients was lower than BMD_{LS} of healthy peers (BMD_{LS}= -0.53SDS, $P=0.01$). However, after correction for bone size, the calculated BMAD_{LS} showed no differences between patients and healthy peers (BMAD_{LS}= -0.21SDS, $P=0.25$).

There was no significant difference in baseline anthro-

pometry, body composition and BM(A)D between non-carriers and carriers of any of the SNPs or haplotypes. In addition, there were no significant differences in age, calcium intake and physical activity.

Body composition during/after therapy

During treatment, the whole group of patients showed an increase in BMI (Δ BMI=+1.43SDS, $P<0.001$). Consequently, BMI became higher than BMI of healthy peers (area under the curve during treatment: $P<0.001$). There was no difference in increase of BMI during treatment between non-carriers and carriers of the different allelic variants (Table 2). After completion of treatment,

Table 1. Genotype distribution in pediatric ALL patients.

VDR 3'-end (BsmI/ApaI/TaqI)				VDR 5'-end (Cdx-2/GATA)				COL1A1 (Sp1)				ESR1 (PvuII/XbaI)				GR (BclI)		
Genotype	Haplotype code	N	%	Genotype	Haplotype code	N	%	Genotype	N	%	Genotype	Haplotype code	N	%	Genotype	N	%	
baT-baT	1/1	17	25	GA-GA	1/1	13	20	GG	49	73	px-px	1,1	20	29	CC	20	41	
baT-BaT	1/2	16	23	GA-GG	1/2	19	30	GT	17	25	px-PX	1,2	29	43	CG	22	45	
baT-bAT	1/3	8	12	GA-AG	1/3	15	23	TT	1	2	px-Px	1,3	4	6	GG	7	14	
baT-BAT	1/4	2	3	GG-GG	2/2	5	8				PX-PX	2,2	10	15				
BaT-BaT	2/2	14	21	GG-AG	2/3	9	14				PX-Px	2,3	4	6				
BaT-bAT	2/3	5	7	AG-AG	3/3	3	5				Px-Px	3,3	1	1				
BaT-BAT	2/4	4	6															
bAT-baT	3/3	2	3															
Total		68				64				67				68		49		
HWE P value		0.30				0.72				0.73				0.84		0.36		

VDR: vitamin D receptor gene; COL1A1: collagen type I α 1 gene; ESR1: estrogen receptor α gene; GR: glucocorticoid receptor gene; N: number; HWE: Hardy Weinberg equilibrium.

Table 2. Change of anthropometry, bone mineral density and body composition for the investigated genetic variations of the VDR, COL1A1, ESR1 and GR genes.

	VDR 3'-end (BsmI/ApaI/TaqI)			VDR 5'-end (Cdx-2/GATA)			COL1A1 (Sp1)			ESR1 (PvuII/XbaI)			GR (BclI)									
	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 1	Haplotype 2	Haplotype 3	Non carrier	Carrier	Carrier							
	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Non carrier	Carrier	Carrier							
Δ : Height (SDS)	-0.56	-0.65	-0.68	-0.57	-0.64	-0.54	-0.87	-0.51**	-0.54	-0.66	-0.56	-0.66	-0.60	-0.68	-0.55	-0.64	-0.65	-0.60	-0.62	-0.64	-0.44	-0.62
BMI (SDS)	1.30	1.51	1.55	1.36	1.49	1.24	0.99	1.48	1.39	1.33	1.58	1.05	1.50	1.36	1.23	1.50	1.78	1.24	1.38	1.81	1.11	1.48
BMD _{LS} (SDS)	0.17	-0.32	-0.33	-0.03	-0.08	-0.44	-0.36	-0.19	-0.15	-0.29	-0.08	-0.40	-0.04	-0.44	-0.57	-0.03	-0.14	-0.16	-0.02	-1.09	-0.18	0.06
BMAD _{LS} (SDS)	-0.01	-0.40	-0.28	-0.25	-0.34	0.01	-0.48	-0.26	-0.39	-0.22	-0.11	-0.57	-0.26	-0.28	-0.50	-0.19	-0.29	-0.25	-0.20	-0.64	-0.24	0.03
BMD _{TB} (SDS)	-0.68	-1.20	-1.33	-0.78	-0.97	-1.15	-1.06	-1.08	-0.99	-1.17	-0.96	-1.23	-0.96	-1.13	-1.62	-0.82	-0.76	-1.13	-0.91	-1.72	-1.40	-0.80
%fat _{TB} (SDS)	1.09	1.50	1.51	1.25	1.31	1.50	0.59	1.47**	1.25	1.39	1.76	0.77*	1.44	1.13	1.13	1.44	1.61	1.22	1.36	1.40	1.47	1.15
LBM (SDS)	0.09	-0.40	-0.46	-0.06	-0.20	-0.34	-0.34	-0.20	-0.07	-0.45	-0.25	0.24	-0.19	-0.30	-0.32	-0.20	-0.12	-0.28	-0.22	-0.36	-0.33	-0.10
Δ 2: Height (SDS)	0.42	0.16	0.24	0.26	0.14	0.56**	0.61	0.10*	0.09	0.36	0.28	0.20	0.14	0.30	0.00	0.32	0.19	0.29	0.28	0.04	0.29	0.27
BMI (SDS)	-0.13	-0.45	-0.34	-0.33	-0.43	-0.02	-0.23	-0.38	-0.30	-0.38	-0.58	0.00+	-0.35	-0.26	-0.29	-0.35	-0.42	-0.28	-0.28	-0.76	-0.01	-0.19
BMD _{LS} (SDS)	-0.01	0.02	-0.02	0.02	-0.06	0.24	-0.02	0.02	-0.02	0.04	-0.12	0.17	0.09	-0.17	0.37	-0.09**	-0.11	0.07	0.00	0.02	-0.09	0.11
BMAD _{LS} (SDS)	-0.17	-0.23	-0.20	-0.21	-0.17	-0.31	-0.36	-0.15	-0.11	-0.31	-0.31	-0.11	-0.06	-0.55**	-0.11	-0.24	-0.25	-0.20	-0.23	0.12	-0.24	-0.23
BMD _{TB} (SDS)	0.44	0.22	0.21	0.37	0.25	0.45	0.54	0.23	0.32	0.30	0.18	0.46	0.36	0.15	0.50	0.24	0.27	0.31	0.27	0.55	0.15	0.36
%fat _{TB} (SDS)	-0.32	-0.79	-0.63	-0.63	-0.73	-0.27	-0.66	-0.66	-0.49	-0.81	-0.85	-0.42	-0.54	-0.84	-0.32	-0.73	-0.81	-0.55	-0.61	-0.88	-0.55	-0.46
LBM (SDS)	0.23	0.22	0.26	0.18	0.12	0.57**	0.61	0.10**	0.03	0.42**	0.11	0.38	0.16	0.37	0.22	0.23	0.11	0.29	0.28	-0.55*	0.38	0.28

VDR: vitamin-D receptor gene; COL1A1: collagen type I α 1 gene; ESR1: estrogen receptor α gene; GR: glucocorticoid receptor gene; Δ 1: change during treatment; Δ 2=change after treatment discontinuation; SDS: standard deviation score; BMI: body mass index; BM(A)DLS: bone mineral (apparent) density of the lumbar spine; BMD_{TB}: bone mineral density of the total body; %fat_{TB}: percentage of fat of the total body; LBM: lean body mass. Values are expressed as mean Δ SDS. Difference between non-carriers and carriers: * $P\geq 0.01$ and ** $0.01<P<0.05$ (ANOVA).

BMI of the patients decreased (Δ BMI=-0.31SDS, $P<0.01$), but remained higher than BMI of healthy peers one year after completion of treatment (BMI=+0.60SDS, $P<0.001$). Furthermore, there was no influence of the carrier status of any of the genotypes on change of BMI after treatment. There were no differences between non-carriers and carriers of the investigated risk alleles in the areas under the curves of BMI either during treatment or during the year after treatment.

During treatment, %fat_{TB} in the patient group was higher than in healthy peers (area under the curve during treatment: $P<0.001$) and increased significantly (Δ %fat_{TB}=+1.32SDS, $P<0.001$). After completion of treatment, %fat_{TB} in the whole study group decreased (Δ %fat_{TB}=-0.60SDS, $P<0.001$), but remained higher than in healthy peers (%fat_{TB}=+0.64SDS, $P<0.001$). A significant difference in gain of %fat_{TB} during treatment was found between non-carriers and carriers of the *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 (non-carriers: Δ %fat_{TB}=+1.76SDS, carriers Δ %fat_{TB}=+0.77 SDS; $P<0.001$) (Table 2). This difference in fat gain between both groups was not evident in the first eight months of treatment, but became obvious during the remaining part of the treatment. No differences in Δ %fat_{TB} between non-carriers and carriers of any of the other investigated risk alleles were found. Furthermore, Δ %fat_{TB} of the non-carriers of the investigated SNPs/ haplotypes was similar to that of the carriers. There was no difference in the areas under the curve of %fat_{TB} between the various carrier groups during treatment or in the year after treatment.

During treatment, the whole group of patients had a lower LBM than healthy peers (area under the curve during treatment: $P<0.001$) and it showed no significant change during treatment. In addition, non-carriers showed the same development of LBM during treatment as carriers of the different risk alleles (Table 2). Although LBM of

the whole group increased in the year after completion of treatment (Δ LBM=+0.23SDS, $P<0.01$), LBM remained lower than in healthy peers (LBM=-0.69SDS, $P<0.001$). The first year after treatment completed, LBM increased in non-carriers of *ESR1* (*PvuII/XbaI*) haplotype 3, but not in carriers (non-carriers: Δ LBM=+0.28SDS, carriers: Δ LBM=-0.55SDS; $P<0.01$). There was no difference in the Δ LBM between non-carriers and carriers of the other investigated risk alleles. During treatment and during the year after treatment, there was no difference in the areas under the curves of LBM between the various carrier groups.

BMD during/after therapy

During treatment, BMD_{LS} of the patients remained lower than that of healthy peers ($P<0.01$). As BMD_{LS} of the whole group did not change either during or after treatment, a year after completion of treatment it was still lower in the patients than in healthy peers (BMD_{LS}=-0.63SDS, $P<0.001$). BMAD_{LS} was only lower in patients than in healthy peers after completion of treatment ($P<0.01$).

Figure 2 shows the effect of different haplotypes of the *VDR* 5'-end (*Cdx-2/GATA*) on BM(A)_{LS}. Carriers of the *VDR* 5'-end haplotype 3 had a lower BMD_{LS} and BMAD_{LS} than non-carriers (area under the curve BMD_{LS}: $P=0.01$, area under the curve BMAD_{LS}: $P=0.03$). There was no difference in BM(A)_{LS} between non-carriers and carriers of haplotype 1 (area under the curve BMD_{LS}: $P=0.68$, area under the curve BMAD_{LS}: $P=0.98$) or haplotype 2 (area under the curve BMD_{LS}: $P=0.91$, area under the curve BMAD_{LS}: $P=0.92$) of the *VDR* 5'-end. No differences in areas under the curves of BM(A)_{LS} were found between non-carriers and carriers of the other investigated risk alleles. Moreover, no differences were shown for Δ BM(A)_{LS} and Δ BM(A)_{LS} between non-carriers and carriers of the

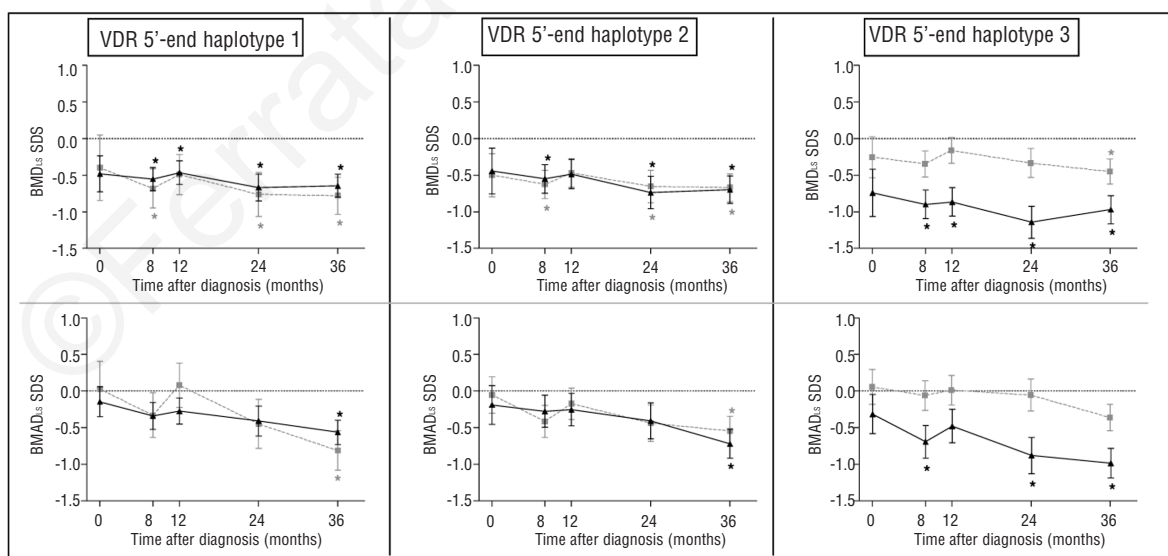


Figure 2. Bone mineral (apparent) density of the lumbar spine in non-carriers versus carriers of haplotypes of the *VDR* 5'-end (*Cdx-2/GATA*) (mean \pm SEM) BM(A)_{LS}: bone mineral (apparent) density of the lumbar spine; SDS: standard deviation score, non-carrier=—■—, carrier=—▲—, * = significant different from 0.

SNPs/ haplotypes (Table 2).

During treatment, BMD_{TB} decreased in patients ($\Delta BMD_{TB} = -1.00SDS$, $P < 0.001$). One year after diagnosis, the total group of patients developed lower levels of BMD_{TB} than healthy peers ($P < 0.01$). During the year after treatment, BMD_{TB} of the patients increased ($\Delta BMD_{TB} = +0.29SDS$, $P < 0.001$), but remained lower than that of healthy peers ($BMD_{TB} = -0.52SDS$, $P < 0.001$). No significant differences in ΔBMD_{TB} or ΔBMD_{TB} between non-carriers and carriers of the *VDR*, *COLIA1*, *ESR1* or *GR* risk alleles were found (Table 2). In addition, there was no difference in areas under the curves of BMD_{TB} between the various carrier groups either during treatment or during the year after completion of treatment.

Fractures

Nine patients sustained a fracture during therapy ($n=5$) or within one year after completion of treatment ($n=4$). Fractures involved the forearm ($n=4$), the tibia ($n=3$), the clavicle ($n=1$) and a vertebra ($n=1$). Except for the vertebral compression fracture, all fractures were preceded by minor trauma. The investigated SNPs/ haplotypes were not associated with an increased fracture risk (Figure 3).

Biomarkers

No differences in serum calcium, PTH and 1,25-dihydroxy-vitamin D were found between non-carriers and carriers of the *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 (Table 3). Moreover, there was no difference in the change of calcium, PTH and 1,25-dihydroxy-vitamin D during therapy and during the year after treatment between non-carriers and carriers of the *VDR* 5'-end haplotype 3.

Discussion

Body composition and polymorphisms

None of the genetic variations in the investigated genes (*VDR*, *COLIA1*, *ESR1* and *GR*) influenced body composition during pediatric ALL treatment, except for haplotype 3 of the *VDR* 5'-promoter region (*Cdx-2/GATA*). Non-carriers of the *VDR* 5'-end haplotype 3 had a larger gain of body fat during treatment than carriers, suggesting a role

for vitamin D in the regulation of body fat during pediatric ALL treatment. The *Cdx-2* A-allele increases *VDR* transcription in the small intestine and may consequently increase calcium absorption.³⁶ Therefore, we hypothesized that non-carriers of the *VDR* 5'-end haplotype 3 (without the *Cdx-2* A-allele) may have relatively lower serum calcium resulting in a relative hyperparathyroidism. This could lead to increased intracellular calcium within adipocytes, inducing lipogenesis.⁴⁶ However, we found no significant differences in serum calcium or PTH between non-carriers and carriers of the *VDR* 5'-end haplotype 3. Therefore, it is questionable whether this mechanism indeed plays a role in regulation of body fat during ALL treatment in children.

The current study suggests that carrying *ESR1* (*PvuII/XbaI*) haplotype 3 negatively influences recovery of LBM after completion of treatment, whereas none of the other genes (*VDR*, *COLIA1* and *GR*) influence body composition after ALL treatment. In adults, polymorphisms in *ESR1* have been described to be associated with measures of adiposity,⁴⁷ although studies on the influence of *ESR1* on LBM are not available. In healthy children, the *PvuII* and *XbaI* SNPs were not related to body composition.³⁰

BMD and polymorphisms

We found a lower BMD_{LS} in patients carrying the *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 than in non-carriers, which was already present at diagnosis. Despite this lower BMD_{LS} , carriers of the *VDR* 5'-end haplotype 3 did not show a larger treatment-related loss of BMD_{LS} . Two previous reports showed that presence of the *Cdx-2* A-allele protected against the loss of BMD and subsequent osteoporotic fractures in elderly individuals.^{8,36} This illustrates that aging may influence the effect of genetic variation on BMD.⁴⁸ Moreover, the present study reports on genotype-based differences in the development of BMD following ALL treatment, while the majority of adult

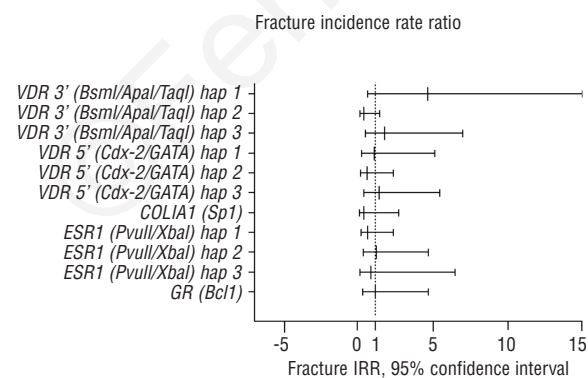


Figure 3. Fracture incidence-rate ratios for non-carriers versus carriers of the different allelic variants. IRR: incidence-rate ratio; *VDR*: vitamin-D receptor gene; *COLIA1*: collagen type I α 1; *ESR1*: estrogen receptor α gene; *GR*: glucocorticoid receptor gene, hap: haplotype.

Table 3. Biochemical markers in non-carriers and carriers of the *VDR* 5'-end haplotype 3.

	VDR 5'-end (<i>Cdx-2/GATA</i>) Haplotype 3		P	P of change
	Non-carrier	Carrier		
Calcium (mmol/L)				
Diagnosis	2.30	2.29	0.87	
Cessation of treatment	2.36	2.36	0.93	$\Delta 1$: 0.84
1 Year after cessation of treatment	2.38	2.37	0.78	$\Delta 2$: 0.81
PTH (SD above upper limit)				
Diagnosis	-2.93	-2.05	0.17	
Cessation of treatment	-1.90	-1.09	0.26	$\Delta 1$: 0.92
1 Year after cessation of treatment	-0.54	-0.52	0.98	$\Delta 2$: 0.27
1,25-dihydroxy-vitamin D (pmol/L)				
Diagnosis	107.2	104.8	0.88	
Cessation of treatment	134.4	128.3	0.70	$\Delta 1$: 0.86
1 Year after cessation of treatment	134.5	145.9	0.25	$\Delta 2$: 0.30

VDR: vitamin-D receptor gene, $\Delta 1$: change during treatment, $\Delta 2$: change after treatment; discontinuation, SD: standard deviation.

reports examined BMD differences between genotypes without chemotherapy.

We did not find any association between *VDR* 3'-end (*BsmI/ApaI/TaqI*) haplotypes and BMD. This is in line with the fact that *VDR BsmI* and *ApaI* SNPs did not influence corticosteroid-induced bone loss in adults receiving corticosteroids for rheumatoid arthritis.⁴⁹ No studies are available on the effects of the *VDR* 3'-end SNPs on BMD in ALL patients treated with corticosteroids. Studies on the relation between BMD and *VDR* 3'-end SNPs in healthy children show conflicting results that may be explained by gene-environment interactions, like dietary calcium intake.^{48,50} In the current study however, calcium intake was adequate with no difference between non-carriers and carriers. Moreover, physical activity could interact with the gene-effect, although there was no difference between non-carriers and carriers. The fact that the individuals in whom the questionnaire was validated had a higher age than our studied ALL patients may have masked a possible gene-physical activity interaction.

We found no influence of polymorphisms of the *COLIA1* (*Sp1*), *ESR1* (*PvuII/XbaI*) and *GR* (*BclI*) on BM(A)D. Because the number of included patients was relatively low, validation of the results in larger cohorts is recommended to confirm our results and to exclude the risk of false-negative findings. So far, no studies on the influence of genetic variation of the *COLIA1*, *ESR1* and *GR* on BM(A)D have been performed in pediatric ALL patients. Several studies in the elderly reported an association between the *Sp1* polymorphism and a lower BMD and increased fracture risk.^{7,21} In pediatric populations this association is less clear. A lower BMD in healthy children carrying the *Sp1* T-allele has been reported but this was mainly due to differences in bone size.^{19,23} The effect of the *ESR1* polymorphism on steroid-induced bone loss has not been previously described. Regarding the *GR* SNPs, the *N363S* was associated with BMD in healthy adults.³²

Our study included no homozygous and 3 heterozygous carriers of the *N363S* SNP, so no conclusion could be drawn on the effects of this SNP on BMD in pediatric ALL.

Fractures

The evaluated risk alleles did not influence fracture risk in our cohort of pediatric ALL patients. This lack of association might be explained by other factors contributing to fracture risk, like an increased tendency to fall due to vincristine neuropathy during ALL treatment.

Conclusion

This is the first study investigating the influence of genetic variation of the *VDR*, *COLIA1*, *ESR1* and *GR* on body composition, BMD and fracture risk in pediatric ALL. We found the *VDR* 5'-end (*Cdx-2/GATA*) haplotype 3 as a protective factor for excessive fat gain during therapy. Moreover, this haplotype 3 of the *VDR* 5'-promoter was determined as a risk factor for a lower BM(A)D_{LS} at diagnosis, which remained a risk factor for a lower BM(A)D_{LS} over the course of ALL treatment. Carriage of *ESR1* (*PvuII/XbaI*) haplotype 3 negatively influenced recovery of LBM after completion of treatment.

Authorship and Disclosures

MLW collected data, performed statistical analysis, interpreted data and wrote the manuscript. RDB collected and interpreted data and wrote the manuscript. SMPFM designed research, interpreted data and supervised the manuscript. AGU performed genetic analyses and supervised the manuscript. WCJH supervised the statistical analysis and interpreted data. RP and MMH designed research, interpreted data and supervised the manuscript.

The authors reported no potential conflicts of interest.

References

- Veerman AJ, Hahlen K, Kamps WA, Van Leeuwen EF, De Van GA, Solbu G, et al. High cure rate with a moderately intensive treatment regimen in non-high-risk childhood acute lymphoblastic leukemia. Results of protocol ALL VI from the Dutch Childhood Leukemia Study Group. *J Clin Oncol*. 1996;14(3):911-8.
- Boot AM, van den Heuvel-Eibrink MM, Hahlen K, Krenning EP, de Muinck Keizer-Schrama SM. Bone mineral density in children with acute lymphoblastic leukaemia. *Eur J Cancer*. 1999;35(12):1693-7.
- Crofton PM, Ahmed SF, Wade JC, Stephen R, Elmlinger MW, Ranke MB, et al. Effects of intensive chemotherapy on bone and collagen turnover and the growth hormone axis in children with acute lymphoblastic leukemia. *J Clin Endocrinol Metab*. 1998; 83(9):3121-9.
- van der Sluis IM, van den Heuvel-Eibrink MM, Hahlen K, Krenning EP, de Muinck Keizer-Schrama SM. Altered bone mineral density and body composition, and increased fracture risk in childhood acute lymphoblastic leukemia. *J Pediatr*. 2002; 141(2):204-10.
- Reid IR. Glucocorticoid osteoporosis--mechanisms and management. *Eur J Endocrinol*. 1997;137(3):209-17.
- Ragab AH, Frech RS, Vietti TJ. Osteoporotic fractures secondary to methotrexate therapy of acute leukemia in remission. *Cancer*. 1970;25(3):580-5.
- Alvarez-Hernandez D, Naves M, Diaz-Lopez JB, Gomez C, Santamaria I, Cannata-Andia JB. Influence of polymorphisms in *VDR* and *COLIA1* genes on the risk of osteoporotic fractures in aged men. *Kidney Int Suppl*. 2003;(85):S14-8.
- Fang Y, van Meurs JB, Bergink AP, Hofman A, van Duijn CM, van Leeuwen JP, et al. *Cdx-2* polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. *J Bone Miner Res*. 2003;18(9): 1632-41.
- Fang Y, van Meurs JB, d'Alesio A, Jhamai M, Zhao H, Rivadeneira F, et al. Promoter and 3'-untranslated-region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: the Rotterdam study. *Am J Hum Genet*. 2005;77(5):807-23.
- Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. *Nature*. 1994;367(6460):284-7.
- Tokita A, Matsumoto H, Morrison NA, Tawa T, Miura Y, Fukamauchi K, et al. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. *J Bone Miner Res*. 1996; 11(7):1003-9.
- Uitterlinden AG, Pols HA, Burger H, Huang Q, Van Daele PL, Van Duijn CM, et al. A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. *J Bone Miner Res*. 1996;11(9):1241-8.
- Gong G, Stern HS, Cheng SC, Fong N, Mordeson J, Deng HW, et al. The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporos Int*. 1999;9(1):55-64.
- Lorentzon M, Lorentzon R, Nordstrom P. Vitamin D receptor gene polymorphism is related to bone density, circulating osteocalcin, and parathyroid hormone in healthy adolescent girls. *J Bone Miner Metab*. 2001;19(5):302-7.
- Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. *N Engl J Med*. 1997;337(2):77-82.
- Baroncelli GI, Federico G, Bertelloni S, Ceccarelli C, Cupelli D, Saggese G.

- Vitamin-D receptor genotype does not predict bone mineral density, bone turnover, and growth in prepubertal children. *Horm Res.* 1999;51(3):150-6.
17. van der Sluis IM, de Muinck Keizer-Schrama SM, Krenning EP, Pols HA, Uitterlinden AG. Vitamin D receptor gene polymorphism predicts height and bone size, rather than bone density in children and young adults. *Calcif Tissue Int.* 2003;73(4):332-8.
 18. Grundberg E, Brandstrom H, Ribom EL, Ljunggren O, Mallmin H, Kindmark A. Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. *Eur J Endocrinol.* 2004;150(3):323-8.
 19. Garnero P, Borel O, Grant SF, Ralston SH, Delmas PD. Collagen Ialpha1 Sp1 polymorphism, bone mass, and bone turnover in healthy French premenopausal women: the OFELY study. *J Bone Miner Res.* 1998;13(5):813-7.
 20. Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP, et al. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest.* 2001;107(7):899-907.
 21. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FE, Grant SF, et al. Relation of alleles of the collagen type Ialpha1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. *N Engl J Med.* 1998;338(15):1016-21.
 22. Suuriniemi M, Kovanen V, Mahonen A, Alen M, Wang Q, Lyytikainen A, et al. COL1A1 Sp1 polymorphism associates with bone density in early puberty. *Bone.* 2006;39(3):591-7.
 23. van der Sluis IM, de Muinck Keizer-Schrama SM, Pols HA, Lequin MH, Krenning EP, Uitterlinden AG. Collagen Ialpha1 polymorphism is associated with bone characteristics in Caucasian children and young adults. *Calcif Tissue Int.* 2002;71(5):393-9.
 24. Berg JP, Lehmann EH, Stakkestad JA, Haug E, Halse J. The Sp1 binding site polymorphism in the collagen type I alpha 1 (COL1A1) gene is not associated with bone mineral density in healthy children, adolescents, and young adults. *Eur J Endocrinol.* 2000;143(2):261-5.
 25. Willing MC, Torner JC, Burns TL, Janz KF, Marshall T, Gilmore J, et al. Gene polymorphisms, bone mineral density and bone mineral content in young children: the Iowa Bone Development Study. *Osteoporos Int.* 2003;14(8):650-8.
 26. Albagha OM, McGuigan FE, Reid DM, Ralston SH. Estrogen receptor alpha gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. *J Bone Miner Res.* 2001;16(1):128-34.
 27. Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, et al. Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. *Jama.* 2004;292(17):2105-14.
 28. van Meurs JB, Schuit SC, Weel AE, van der Klift M, Bergink AP, Arp PP, et al. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet.* 2003;12(14):1745-54.
 29. Boot AM, van der Sluis IM, de Muinck Keizer-Schrama SM, van Meurs JB, Krenning EP, Pols HA, et al. Estrogen receptor alpha gene polymorphisms and bone mineral density in healthy children and young adults. *Calcif Tissue Int.* 2004;74(6):495-500.
 30. Tobias JH, Steer CD, Vilarino-Guell C, Brown MA. Effect of an estrogen receptor-alpha intron 4 polymorphism on fat mass in 11-year-old children. *J Clin Endocrinol Metab.* 2007;92(6):2286-91.
 31. van Rossum EF, Koper JW, van den Beld AW, Uitterlinden AG, Arp P, Ester W, et al. Identification of the Bcl polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. *Clin Endocrinol (Oxf).* 2003;59(5):585-92.
 32. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, et al. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. *J Clin Endocrinol Metab.* 1998;83(1):144-51.
 33. van Schoor NM, Dennison E, Lips P, Uitterlinden AG, Cooper C. Serum fasting cortisol in relation to bone, and the role of genetic variations in the glucocorticoid receptor. *Clin Endocrinol (Oxf).* 2007; 67(6):871-8.
 34. Rosmond R, Chagnon YC, Holm G, Chagnon M, Perusse L, Lindell K, et al. A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis. *Obes Res.* 2000; 8(3):211-8.
 35. Veerman AJP, Kamps WA, van den Berg-de Ruitter E, van den Berg H, Bökkerink JPM, Bruin MCA, et al. Dexamethasone based therapy in childhood acute lymphoblastic leukaemia with favourable event-free survival: results of the Dutch Childhood Oncology Group (DCOG) ALL-9 protocol (1997-2004). *The Lancet Oncology* 2009; 10(10):957-66.
 36. Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, et al. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. *J Bone Miner Res.* 2001;16(7):1256-64.
 37. Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nat Genet.* 1996;14(2):203-5.
 38. Tissing WJ, Meijerink JP, den Boer ML, Brinkhof B, van Rossum EF, van Wering ER, et al. Genetic variations in the glucocorticoid receptor gene are not related to glucocorticoid resistance in childhood acute lymphoblastic leukemia. *Clin Cancer Res.* 2005;11(16):6050-6.
 39. Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP. Body index measurements in 1996-7 compared with 1980. *Arch Dis Child.* 2000;82(2):107-12.
 40. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-23.
 41. Kroger H, Vainio P, Nieminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. *Bone.* 1995;17(2):157-9.
 42. van der Sluis IM, de Ridder MA, Boot AM, Krenning EP, de Muinck Keizer-Schrama SM. Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults. *Arch Dis Child.* 2002 Oct;87(4):341-7; discussion 341-7.
 43. Kemper HC, Bakker I, Twisk JW, van Mechelen W. Validation of a physical activity questionnaire to measure the effect of mechanical strain on bone mass. *Bone.* 2002;30(5):799-804.
 44. Angus RM, Sambrook PN, Pocock NA, Eisman JA. A simple method for assessing calcium intake in Caucasian women. *J Am Diet Assoc.* 1989;89(2):209-14.
 45. Roodnat JJ, van Gurp EA, Mulder PG, van Gelder T, de Rijke YB, de Herder WW, et al. High pretransplant parathyroid hormone levels increase the risk for graft failure after renal transplantation. *Transplantation.* 2006;82(3):362-7.
 46. McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. *Med Hypotheses.* 2003;61(5-6):535-42.
 47. Fox CS, Yang Q, Cupples LA, Guo CY, Atwood LD, Murabito JM, et al. Sex-specific association between estrogen receptor-alpha gene variation and measures of adiposity: the Framingham Heart Study. *J Clin Endocrinol Metab.* 2005;90(11):6257-62.
 48. Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP. Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res.* 1998;13(3):363-70.
 49. Ho YV, Briganti EM, Duan Y, Buchanan R, Hall S, Seeman E. Polymorphism of the vitamin D receptor gene and corticosteroid-related osteoporosis. *Osteoporos Int.* 1999;9(2):134-8.
 50. Salamone LM, Glynn NW, Black DM, Ferrell RE, Palermo L, Epstein RS, et al. Determinants of premenopausal bone mineral density: the interplay of genetic and lifestyle factors. *J Bone Miner Res.* 1996;11(10):1557-65.