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 (Δ%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.

Citation: te Winkel ML,1 van Beek RD, de Muinck Keizer-Schrama SMPF, Uitterlinden AG, Hop WCJ, Pieters R, and van den Heuvel-Eibrink MM. Pharmacogenetic risk factors for altered bone mineral density and body composition in pediatric acute lymphoblastic leukemia. Haematologica 2010;95:752-759. doi:10.3324/haematol.2009.016303

Acknowledgments: the authors would like to thank Y. de Rijke for her advice on the assessments of biochemical markers and P. Arp and Y. Fang for their technical assistance in SNP genotyping. We would also like to thank A. Darlington for editorial assistance.

Funding: this work was supported by the Pediatric Oncology Foundation Rotterdam (KOCR).

Manuscript received on August 28, 2009. Revised version arrived on October 22, 2009. Manuscript accepted on October 22, 2009.

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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 corticosteroids[5] 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 pre-menopausal 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 (*COLIA1*). 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 *COLIA1* 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 *PvuI* 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 *XbaI 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*, *COLIA1*, *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 50×10^9/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/m^2 (high-risk) and 1,370 mg/m^2 (non-high risk). Total cumulative dose of methotrexate was 13,650 mg/m^2 in the high-risk protocol and 8,100mg/m^2 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 *COLIA1* (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/height^2. 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$_{LS}$). To correct for bone size, we calculated bone mineral apparent density (BMAD) of the lumbar spine with the model

$BMAD_{LS}=BMD_{LS}x(4/(\pi x width))$. '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/ApaI/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*).

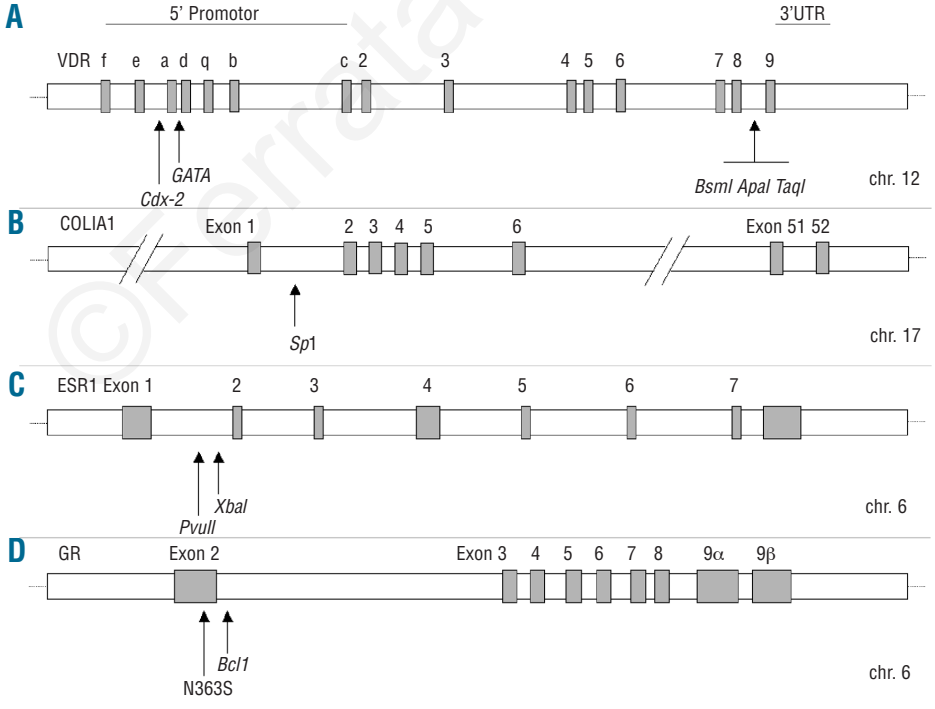

Figure 1. Genomic structure and positions of the single nucleotide polymorphisms investigated in current study. (A) The vitamin-D receptor gene (VDR). (B) The collagen type I α 1 gene (COLIA1). (C) The estrogen receptor α gene (ESR1). (D) The glucocorticoid receptor gene (GR). UTR: untranslated region; 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 anthropometry, 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_1BMI=+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)				COLIA1 (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; COLIA1: 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*, *COLIA1*, *ESR1* and *GR* genes.

	VDR 3'-end (*BsmI/ApaI/TaqI*)						VDR 5'-end (*Cdx-2/GATA*)						COLIA1 (*Sp1*)		ESR1 (*PvuII/XbaI*)						GR (*BclI*)	
	Haplotype 1		Haplotype 2		Haplotype 3		Haplotype 1		Haplotype 2		Haplotype 3				Haplotype 1		Haplotype 2		Haplotype 3			
	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier	Non carrier	Carrier
Δ_1																						
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; COLIA1: collagen type I alpha 1 gene; ESR1: estrogen receptor alpha gene; GR: glucocorticoid receptor gene; Δ1: change during treatment; Δ2=change after treatment discontinuation; SDS: standard deviation score; BMI: body mass index; BM(A)DLS: bone mineral (apparent) density of the lumbar spine; BMDTB: bone mineral density of the total body; %fatTB: percentage of fat of the total body; LBM: lean body mass. Values are expressed as mean ΔSDS. Difference between non-carriers and carriers: *P≥0.01 and **0.01<P<0.05 (ANOVA).*

BMI of the patients decreased (Δ_2BMI=-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_1%fat$_{TB}$=+1.32SDS, P<0.001). After completion of treatment, %fatTB in the whole study group decreased (Δ_2%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_1%fat$_{TB}$=+1.76SDS, carriers Δ_1%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_1%fat$_{TB}$ between non-carriers and carriers of any of the other investigated risk alleles were found. Furthermore, Δ_2%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_2LBM=+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_2LBM=+0.28SDS, carriers: Δ_2LBM=-0.55SDS; P<0.01). There was no difference in the Δ_2LBM 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)D$_{LS}$. 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)D$_{LS}$ 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)D$_{LS}$ were found between non-carriers and carriers of the other investigated risk alleles. Moreover, no differences were shown for Δ_1BM(A)D$_{LS}$ and Δ_2BM(A)D$_{LS}$ between non-carriers and carriers of the

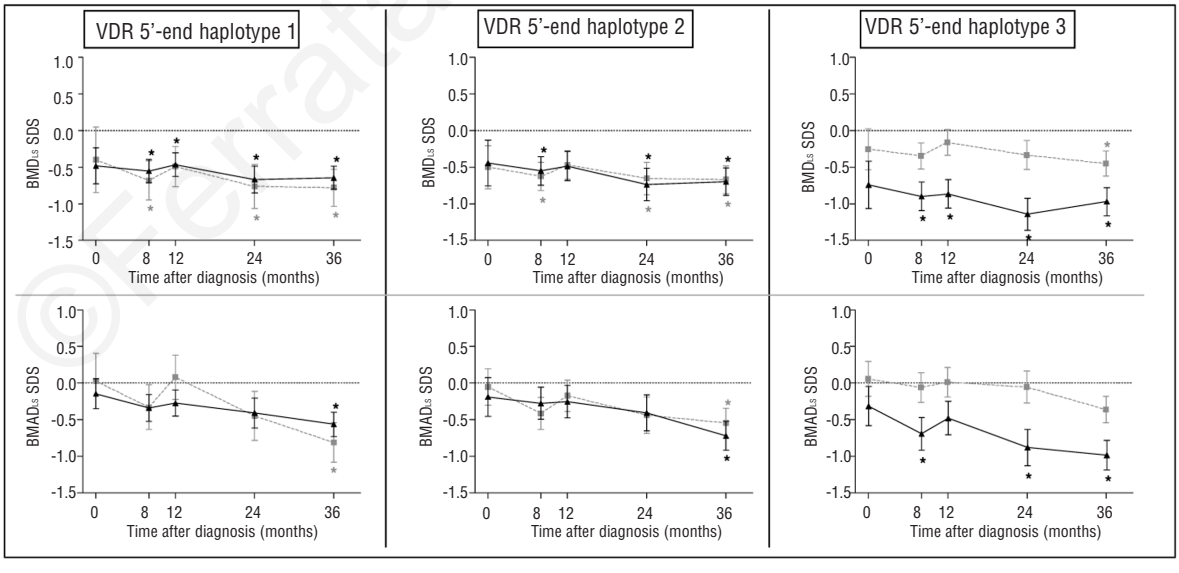

Figure 2. Bone mineral (apparent) density of the lumbar spine in non-carriers versus carriers of haplotypes of the VDR 5'-end (Cdx-2/ GATA) (mean ± SEM) BM(A)D$_{LS}$: bone mineral (apparent) density of the lumbar spine; SDS: standard deviation score, non-carrier=—■—, carrier=–▲–, *=significant different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

SNPs/ haplotypes (Table 2).

During treatment, BMD_{TB} decreased in patients (Δ_1BMD_{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 (Δ_2BMD_{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 Δ_1BMD_{TB} or Δ_2BMD_{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

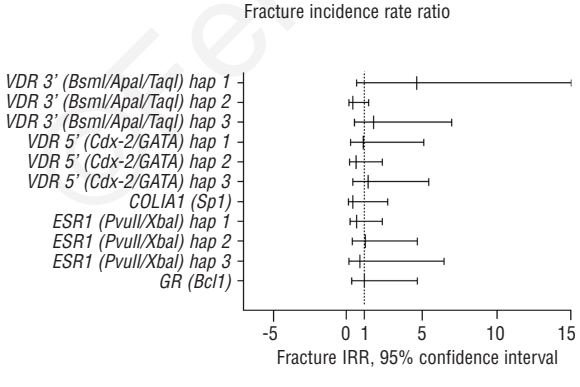

Figure 3. Fracture incidence-rate ratios for non-carriers versus carriers of the different allelic variants. IRR: incidence-rate ratio; VDR: vitamin-D receptor gene; COLIA1: collagen type I α 1; ESR1: estrogen receptor α gene; GR: glucocorticoid receptor gene, hap: haplotype.

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

	VDR 5'-end (Cdx-2/GATA) Haplotype 3			
	Non-carrier	Carrier	*P*	*P* of change
Calcium (mmol/L)				
Diagnosis	2.30	2.29	0.87	
Cessation of treatment	2.36	2.36	0.93	Δ1: 0.84
1 Year after cessation of treatment	2.38	2.37	0.78	Δ2: 0.81
PTH (SD above upper limit)				
Diagnosis	-2.93	-2.05	0.17	
Cessation of treatment	-1.90	-1.09	0.26	Δ1: 0.92
1 Year after cessation of treatment	-0.54	-0.52	0.98	Δ2: 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	Δ1: 0.86
1 Year after cessation of treatment	134.5	145.9	0.25	Δ2: 0.30

VDR: vitamin-D receptor gene, Δ1:change during treatment, Δ2: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.

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