Genetic Variation of the Vitamin D Binding Protein Affects Vitamin D Status and Response to Supplementation in Infants

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Context: Single nucleotide polymorphisms (SNPs) of the vitamin D binding protein encoding the *GC* (group component) gene affect 25-hydroxyvitamin D (25OHD) concentrations, but their influence on vitamin D status and response to vitamin D supplementation in infants is unknown.

Objective: To study *GC* genotype–related differences in 25OHD concentrations and the response to supplementation during a vitamin D intervention study in infants.

Design: In this randomized controlled trial, healthy term infants received vitamin D_3 (10 or 30 μg/d) from 2 weeks to 24 months of age. *GC* SNPs rs2282679, rs4588, rs7041, and rs1155563 were genotyped. rs4588/7041 diplotype and haplotypes of rs2282679, rs4588, and rs7041 ($Haplo_{3SNP}$) and of all four SNPs ($Haplo_{4SNP}$) were determined.

Main Outcome Measures: 25OHD measured in cord blood at birth and at 12 and 24 months during intervention.

Results: A total of 913 infants were included. Minor allele homozygosity of all studied *GC* SNPs, their combined haplotypes, and rs4588/rs7041 diplotype 2/2 were associated with lower 25OHD concentrations at all time points in one or both intervention groups [analysis of covariance (ANCOVA) $P < 0.043$], with the exception of rs7041, which did not affect 25OHD at birth. In the high-dose supplementation group receiving 30 μg/d vitamin D_3, but not in those receiving 10 μg/d, genotype of rs2282679, rs4588, and rs7041; diplotype; and $Haplo_{3SNP}$ significantly affected intervention response (repeated measurement ANCOVA $P_{interaction} < 0.019$). Minor allele homozygotes had lower 25OHD concentrations and smaller increases in 25OHD throughout the intervention.

Conclusions: In infants, vitamin D binding protein genotype affects 25OHD concentration and efficiency of high-dose vitamin D_3 supplementation. ***(J Clin Endocrinol Metab* 104: 5483–5498, 2019)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 15 March 2019. Accepted 25 July 2019.
First Published Online 31 July 2019

Abbreviations: 25OHD, 25-hydroxyvitamin D; ANCOVA, analysis of covariance; DBP, vitamin D binding protein; SDS, standard deviation score; SNP, single nucleotide polymorphism; VIDI, Vitamin D Intervention in Infants.

Vitamin D insufficiency is common worldwide (1). Many countries have implemented recommendations of vitamin D supplementation and vitamin D fortification of food products (2–4). Supplementation is particularly important during infancy and early childhood, when vitamin D supply from diet and sunlight may be scarce and growth and development are rapid. Vitamin D insufficiency in this age group can have lifelong skeletal and possibly extraskeletal effects (1, 5–7).

Concentration of 25-hydroxyvitamin D (25OHD) is an acknowledged marker of vitamin D status. Optimal 25OHD concentration is unclear, but in children concentrations >50 nmol/L are generally considered sufficient. Serum 25OHD concentrations have shown notable individual variation, partly due to genetic factors (8–10). Previous reports and genome-wide association studies have identified the *GC* (group component) gene, encoding the vitamin D binding protein (DBP), as one of the genes associated with differences in 25OHD concentrations and with individual risk of vitamin D insufficiency (8, 11–13). DBP is a 52- to 59-kDa protein of the albumin gene family, which in the circulation binds and transports up to 90% of vitamin D and its metabolites (14, 15). The *GC* gene has been found to be greatly polymorphic, with >120 described variants, some resulting in distinct structural phenotypes of DBP (14, 16). The distribution of these variants differs between ethnic groups (14).

Two of the most studied genetic variants of the *GC* gene, single nucleotide polymorphisms (SNPs) rs4588 [NM_000583.3 (GC): c.1307C>A, p.Thr436Lys] and rs7041 (c.1296T>G, p.Asp432Glu), have been repeatedly shown to be linked to differences in 25OHD concentrations. In adults and older children, associations have been demonstrated for both genotypes of the SNPs and their six diplotypes, reflecting the combinations of the three common phenotypic variants of the DBP (1S, 1F, and 2) (9, 17–19). Among other identified polymorphisms of the *GC* gene, several adult studies have shown the intronic SNPs rs2282679 and rs1155563 to be associated with differences in 25OHD concentrations (8, 11, 20–22). For rs2282679, genotype-related differences in 25OHD concentrations have also been found in infants at birth (23).

In addition to associations with 25OHD concentration, previous studies have shown possible genotype-related differences in response to vitamin D supplementation in adults, including pregnant women (24–27), but these differences have not been studied in children.

Potential associations of the *GC* SNPs with vitamin D supplementation response in infants are unclear. Because 25OHD concentrations and response to vitamin D supplementation show individual variation, the optimal dose for vitamin D supplementation in infants may also be genotype dependent (8, 17, 24, 25, 27, 28). Our study examined how genetic variation in four SNPs of the DBP encoding *GC* gene affects 25OHD concentrations and response to two different vitamin D supplementation doses in infants from 2 weeks to 24 months of age.

Methods

Participants and follow-up

This study is a part of the randomized, double-blind, controlled Vitamin D Intervention in Infants (VIDI) trial; protocol, inclusion, and exclusion criteria of the VIDI trial have been described (29, 30). Ethical approval for the study was granted by the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (107/13/03/03/2012), and the study was performed in accordance with the principles of the Helsinki Declaration. The trial protocol is registered in ClinicalTrials.gov (NCT01723852). Parents of participants gave written informed consent at recruitment.

A total of 987 healthy infants of mothers of Northern European origin, born at term and with birthweight appropriate for gestational age, participated in the VIDI trial performed at the Kätilöopisto Maternity Hospital in Helsinki, Finland, between January 2013 and June 2016. The participants were randomized to receive daily vitamin D_3 supplementation of either 10 μg (400 IU) (Group 10), which is the standard recommended supplementation for this age-group in Finland (4, 31), or 30 μg (1200 IU) (Group 30) from age 2 weeks to 24 months.

Baseline data on infant birth, maternal background, and use of vitamin D supplementation during pregnancy were collected retrospectively from medical records and by questionnaires. Umbilical cord blood samples collected at birth were used for genomic DNA and to assess baseline 25OHD concentrations.

At the 12- and 24-month study visits, venous blood samples were obtained for analyses of 25OHD concentrations, and weight and length of the participants were measured and transformed into standard deviation score (SDS) using Finnish pediatric growth references (32).

Adherence to the intervention D_3 supplement was calculated from study diaries in which administration of supplement was recorded daily by the parents of the participating child. Duration of breastfeeding was also reported in the diaries. The study diaries were collected and reviewed every 3 to 6 months during the trial (29, 30).

VIDI trial participants who were later found not to fulfill the initial inclusion criteria (n = 12), who were diagnosed with basic pathologies (n = 8), or who lacked genotype data were excluded from analyses. The final study cohort included a total of 913 participants with available genotyping results for one or more of four selected *GC* SNPs (rs2282679, rs4588, rs7041, and rs1155563) in addition to baseline data.

Genotype analysis

Genomic DNA was extracted from cord blood samples in the laboratory of the Finnish National Institute for Health and Welfare using automated Chemagen MSM1 extraction (PerkinElmer Inc., Chemagen Technologie GmbH, Baesweiler, Germany) or the Gentra Puregene kit (Qiagen GmgH, Hilden, Germany) in accordance with the manufacturers' instructions.

The studied SNPs were previously selected from the HapMap project database (33), preferring functional polymorphisms with high heterozygosity levels and previously shown associations with 25OHD concentrations (34). Genotyping of SNPs rs2282679, rs4588, rs7041, and rs1155563 was performed using TaqMan Assays (Thermo-Fisher, Waltham, MA) (Taqman SNP Assay ID: C_26407519_10, C_8278879_10, C_3133594_30, and C_8278782_20, respectively) and the qPCR Bio-Rad CFX384 C1000 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA) or the qPCR ABI Prism 7900HT system (Applied Biosystems, Foster City, CA) according to the manufacturers' instructions. Amplification was performed by protocols of 95°C for 3 or 10 minutes, followed by 39 or 40 cycles of 15 seconds at 92°C or 95°C and 1 minute at 60°C, respectively. Results were determined using end-point protocol analysis by CFX Manager 3.1 (BioRad) or SDS 2.3 (Applied Biosystems) software. Previously genotyped samples from adult control subjects (4%) as well as randomly chosen duplicate internal (3%) and negative control subjects (2%) were used to validate the obtained genotyping results.

The obtained genotypes of SNP rs4588/rs7041 were combined into six known diplotypes representing the six structural phenotype variants of the DBP protein (1S/1S, 1S/1F, 1F/1F, 1S/2, 1F/2, 2/2) (9, 35). The genotypes of the studied SNPs were also combined into haplotypes including all four ($Haplo_{4SNP}$) and three ($Haplo_{3SNP}$) (excluding rs1155563) SNPs. Haplotypes were determined, and linkage disequilibrium (LD) and Hardy-Weinberg equilibrium were evaluated using Haploview 4.2 (Broad Institute, Boston, MA) software. Haplotype homozygotes were identified and used in the analyses.

Biochemical analyses

Concentrations of 25OHD at baseline (cord blood) and at 12 and 24 months were analyzed at the Pediatric Research Center, University of Helsinki, using a fully automated IDS-iSYS immunoassay system with chemiluminescence detection (Immunodiagnostic Systems Ltd., Bolton, UK). As previously reported (30), cord plasma 25OHD concentrations were adjusted to be comparable with serum 25OHD concentrations and further corrected due to changes in the IDS-iSYS system, in accordance with the manufacturers' instructions.

Intra-assay variation for 25OHD concentrations was <13% for cord blood and <5% for the 12- and 24-month samples. The quality and accuracy of the used analyses were validated by participation in the vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, London, UK). The method used showed a <8% positive bias when compared with the National Institute of Standards and Technology Reference Measurement Procedure.

Statistical methods

Results are given as means and SD or as 95% CIs for adjusted means. The normality of distribution within variables was visually evaluated. Logarithmic conversion was used for non-normally distributed variables. Differences in normally distributed variables were studied using independent samples *t* test, and Mann-Whitney *U* test was used when normal distribution was not obtained by logarithmic conversion. The χ^2 test was used for comparisons of categorical variables between intervention groups.

ANOVA and analysis of covariance (ANCOVA) were used to evaluate the impact of SNP genotypes, diplotypes, and haplotype homozygotes on serum 25OHD concentrations at birth and at 12 and 24 months. Maternal and infant-related factors showing significant independent associations with S25OHD concentrations (maternal vitamin D supplementation during pregnancy, season, length-adjusted weight SDS, duration of breastfeeding, intervention group, and adherence to intervention vitamin D_3 supplementation) were used as covariates. Bonferroni or Tamhane adjustments were used for multiple comparisons. Linear regression analysis was used to evaluate mean allelic effect on 25OHD concentration of the studied polymorphisms.

Temporal change in 25OHD concentration during the intervention and modifying effects of the studied SNPs were analyzed using linear mixed models for repeated measurements (repeated measurements ANCOVA) including all three time points (baseline and 12 and 24 months). Analyses were performed for all participants and, because the intervention group showed significant interaction with temporal change of 25OHD, separately within intervention groups. Nongenetic factors affecting temporal change of 25OHD concentration (season of birth, length-adjusted weight SDS at 24 months, duration of breastfeeding, adherence to intervention vitamin D_3 supplementation, and interaction between adherence and temporal change) were used as covariates. Because duration of breastfeeding was a significant covariate only in Group 10, it was not included in the model when analyzing the higher-dose intervention group (Group 30). To further evaluate intervention response, analyses were performed in the subset of participants with >80% adherence to intervention D_3 supplement.

In participants with adherence >80%, the mean changes in 25OHD concentrations at 24 months of intervention (Δ25OHD = 25OHD concentration at 24 months − baseline 25OHD concentration) by genotype, diplotype, and haplotype were calculated and evaluated by ANOVA and ANCOVA, adjusting for season of birth, length-adjusted weight SDS at 24 months, and adherence to supplementation. For variables with variances that were not equal, the Welch test of equality of means was used to further evaluate differences between variants.

SPSS Statistics 24 (IBM, Armonk, NY) software was used for data analyses. A *P* value <0.05 was considered statistically significant. Missing values were excluded analysis-by-analysis.

Results

Participants and distributions of genotypes, diplotypes, and haplotypes

A total of 913 infants (49.7% girls) were included in this study. Participant details are described in Table 1. Baseline characteristics did not differ between intervention groups.

Genotype call rates varied between 92% and 99%, with consistent negative and positive controls. Genotype was determined for all four studied SNPs in 89% of participants. The distributions of the studied genotypes were in line with available previously reported genotype data (36–38). The obtained genotyping results were in Hardy-Weinberg equilibrium. The studied SNPs were in strong linkage disequilibrium ($r^2 > 0.8$).

Three different combinations of haplotype homozygotes were identified for the haplotype, including three SNPs (rs2282679, rs4588, and rs7041; $Haplo_{3SNP}$) (*TGC*, combined major alleles; *TGA* and *GTA*, combined minor alleles) and for the haplotype including all four studied SNPs ($Haplo_{4SNP}$) (*TGCT*, combined major alleles; *TGAT* and *GTAC*, combined minor alleles). Genotype, diplotype, and haplotype distributions (Table 2) differed in the two intervention groups.

Biochemical variables

In accordance with the previously described outcomes of the VIDI trial (30), the mean 25OHD concentrations did not differ between intervention groups at baseline but were significantly higher in Group 30 at 12 and 24 months of intervention (Table 1). The majority of participants (>95.7%) were vitamin D sufficient throughout the trial, with a 25OHD concentration >50 nmol/L. Concentrations of 25OHD were, at all time points, lowest in spring when compared with other seasons (ANOVA $P < 0.050$).

Associations of genotypes, diplotypes, and haplotypes with 25OHD concentrations

Adjusted mean serum 25OHD concentrations by genotype, diplotype, and haplotype and results for analyses of covariance during follow-up are presented in Table 3. Table 4 shows adjusted mean allelic effect sizes for genotypes and the effect of diplotype and haplotypes, with results for multivariate linear regression.

SNPs rs2282679, r4588, and rs1155563 were associated with 25OHD concentrations at all time points (Table 3). Common (major) allele homozygotes had the highest and rare (minor) allele homozygotes the lowest 25OHD concentrations. rs7041 major allele homozygotes showed significantly higher 25OHD concentrations than minor allele homozygotes in Group 10 at 12 months and in both intervention groups at 24 months. Mean allelic effect size per one minor allele in the studied SNPs varied between −3.8 and −10.8 nmol/L, being greatest for rs2282679 (Table 4).

Diplotype and $Haplo_{3SNP}$ affected 25OHD concentrations at all studied time points. $Haplo_{4SNP}$ was associated with 25OHD concentrations at 12 months in both intervention groups and in Group 10 at 24 months (Table 3). Major allele homozygote haplotypes and diplotype 1 (*1S/1S*, *1F/1S*, and *1F/1F*) had the highest 25OHD concentrations, and minor allele homozygote haplotypes and diplotype 2 (*1S/2*,*1F/S*, and *2/2*) had the lowest 25OHD concentrations. Mean effect size of diplotype 2 vs 1 ranged from −4.4 nmol/L at baseline to −10.9 nmol/L at 24 months (Table 4). When comparing minor with major allele homozygotes of $Haplo_{3SNP}$ and $Haplo_{4SNP}$, the mean effect size ranged from −12.6 to −33.7 nmol/L and was significant at baseline and at 12 months in both intervention groups and in Group 10 at 24 months.

Temporal change of 25OHD concentration and genotype, diplotype, and haplotype

When examining the effects of genetic variants on temporal 25OHD change in a model including concentrations at baseline and at 12 and 24 months, we found mean adjusted 25OHD concentrations to differ between genotype, diplotype, and haplotype in both intervention groups ($P_{variant}$), but in Group 10 these did not significantly affect intervention response. In contrast, in the intervention group receiving higher vitamin D_3 supplementation (Group 30), we observed a significant interaction between variants and temporal change ($P_{interaction} < 0.019$), indicating differences in intervention response between variants of rs2282679, rs4588, and rs7041, diplotype, and $Haplo_{3SNP}$ (Table 5). Minor allele homozygotes, diplotype 2/2, and haplotype homozygotes for the combination of minor alleles showed the smallest temporal increases in 25OHD. Differences in temporal change for $Haplo_{4SNP}$ were not statistically significant. Temporal change and results by intervention group and genotypes or diplotype and haplotypes are presented in Fig. 1 and Fig. 2, respectively.

When including only study subjects with >80% adherence to vitamin D_3 supplementation, the genotypes of rs2282679, rs4588, rs7041, and diplotype were significantly associated with differences in temporal 25OHD changes in Group 30 ($P_{interaction} < 0.028$), but the associations for $Haplo_{3SNP}$ no longer reached significance ($P_{interaction} = 0.180$).

In accordance with the results for temporal change, the calculated mean change of 25OHD concentration (Δ25OHD) from baseline to 24 months of intervention, in participants with adherence >80%, differed significantly between genotypes of rs2282679, rs4588, rs7041, and diplotype in Group 30 (Table 6). Significant differences in Δ25OHD ranged from 13 to 17 nmol/L between major and minor homozygotes and 15 nmol/L between diplotypes *1S/1S* and *1S/2*. For haplotypes, differences in Δ25OHD of up to 20 nmol/L between minor and major allele homozygotes were observed but reached significance only for unadjusted means of $Haplo_{3SNP}$ (unequal variances, Welch test for equality of means $P = 0.008$, Tamhane adjusted $P = 0.008$). In Group 10, Δ25OHD was small and did not significantly differ between genotypes, but in both intervention groups Δ25OHD was greatest in major allele homozygotes and smallest for genotypes and haplotypes of minor allele homozygotes. Figure 3 presents adjusted mean Δ25OHD from baseline to 24 months in both intervention groups by genotype, in participants

Table 1. Characteristics of Study Participants

	All	Group 10	Group 30	P^a
Baseline				
Participants, n (% girls)	913 (49.7)	459 (49.7)	454 (49.8)	0.974
Duration of gestation, wk	40.2 (1.1)	40.1 (1.1)	40.3 (1.1)	0.076
Weight at birth, kg	3.5 (0.4)	3.5 (0.4)	3.6 (0.4)	0.058
Length-adjusted weight at birth, SDS	0.1 (1.0)	0.1 (0.9)	0.1 (1.0)	0.240
Maternal vitamin D supplement during pregnancy, μg/d	15.3 (15.5)	16.1 (17.8)	14.5 (12.9)	0.147
12-mo follow-up				
Participants, n (% girls)	816 (50.7)	409 (51.1)	407 (50.4)	0.834
Weight at 12 mo, kg	9.8 (1.1)	9.8 (1.2)	9.8 (1.2)	0.359
Length-adjusted weight at 12 mo, SDS	0.0 (1.0)	−0.0 (1.0)	0.0 (1.0)	0.911
Adherence 0–12 mo, %	89.2 (11.4)	89.3 (11.8)	89.1 (10.9)	0.570
Adherence 0–12 mo >80%, %	84.9	86.1	83.5	0.306
24-mo follow-up				
Participants, n (% girls)	776 (50.3)	384 (50.3)	392 (50.3)	0.999
Weight at 24 mo, kg	12.5 (1.4)	12.5 (1.3)	12.6 (1.4)	0.317
Length-adjusted weight at 24 mo, SDS	−0.1 (1.0)	−0.1 (1.0)	0.0 (1.0)	0.084
Duration of breastfeeding, mo	10.7 (5.6)	10.5 (5.7)	10.9 (5.5)	0.285
Adherence 0–24 mo, %	88.0 (12.6)	88.7 (11.8)	87.3 (13.4)	0.349
Adherence 0–24 mo >80%, %	84.0	86.4	81.6	0.070
25OHD concentration				
At baseline (cord blood), nmol/L	81.3 (25.9)	81.4 (27.8)	81.2 (23.8)	0.883
At 12 mo, nmol/L	98.6 (28.8)	82.7 (20.0)	114.4 (27.6)	<0.001
At 24 mo, nmol/L	102.4 (27.8)	86.7 (19.8)	117.8 (25.8)	<0.001

Values are reported as means and SD unless otherwise noted.

[a]Independent samples *t* test for analyses of differences between intervention groups for anthropometric and biochemical variables. Pearson χ^2 for number of participants. Mann-Whitney *U* test for adherence. Number of subjects if data available for <95% at follow-up: data on maternal vitamin D supplementation during pregnancy, n = 813; data on serum 25OHD concentration at 12 mo, n = 757.

with >80% adherence to intervention D_3 supplementation, and results for ANCOVA.

Discussion

The results of this randomized controlled trial in infants show that vitamin D binding protein genotype affects vitamin D status and response to vitamin D supplementation. The key findings of this study are that in infants aged 24 months and younger, individual variation of the *GC* gene not only affects 25OHD concentrations from birth onward but also modifies temporal changes in 25OHD concentrations in response to high-dose vitamin D_3 supplementation. In our intervention group receiving 30 μg/d of vitamin D_3, participants homozygous for minor alleles of SNPs rs2282679, rs4588, rs7041, combined minor allele haplotype, and participants with DBP phenotype 2 (*GC* diplotype *1S/2*, *1F/2*, and *2/2*) had the lowest 25OHD concentrations and showed the smallest increase in 25OHD concentrations throughout the intervention. Participants homozygous for the major alleles of these SNPs and their haplotype, as well as those with the DBP phenotype 1 (*GC* diplotype *1S/1S*, *1S/1F*, and *1F/1F*), showed higher 25OHD concentrations and greater intervention response.

Our findings support the previously reported cross-sectional associations of *GC* SNP genotype and rs4588/rs7041 diplotype with 25OHD concentrations in adults and children, linking minor alleles of rs2282679, rs4588, rs7041, rs1155563, and rs4588/rs7041 diplotype 2 with lower 25OHD concentrations (8, 11, 18, 21, 23, 39, 40). In our study population these associations are evident at birth, not only for rs2282679 as previously shown (23) but also for variants of rs4588, rs1155563, and rs4588/rs7041 diplotype. We also found that combined homozygote carriers of the minor alleles of SNPs rs2282679, rs4588, and rs7041 ($Haplo_{3SNP}$) and rs1155563 ($Haplo_{4SNP}$) show the lowest 25OHD concentrations during the intervention.

Research on *GC* genotype–related differences in response to vitamin D supplementation is scarce and inconclusive, especially in a randomized controlled trial setting, and studies in young children are lacking. In adult populations, one study has reported the minor allele rs4588 genotype to be linked with a greater increase of 25OHD in response to vitamin D supplementation (28), whereas others reported no significant differences in dose response between rs4588 and rs7041 genotypes in adults aged 45 to 75 years (41) or 60 to 84 years (42) or in postmenopausal women (43). On the other hand, rs4588 major allele carriers have been reported to show greater

Table 2. Genotype, Diplotype, and Haplotype Distributions and Results for Analyses of Differences in Distributions Between Intervention Groups

	Variant	All, n (%)	Group 10, n (%)	Group 30, n (%)	P^a
rs2282679 (genotyped n = 889)	*TT*	571 (64.2)	267 (60.3)	304 (68.2)	0.009
	GT	283 (31.8)	152 (34.3)	131 (29.4)	
	GG	35 (3.9)	24 (5.4)	11 (5.4)	
rs4588 (genotyped n = 893)	*GG*	568 (63.6)	266 (59.8)	302 (67.4)	0.025
	GT	290 (32.5)	156 (35.1)	134 (29.9)	
	TT	35 (3.9)	23 (5.2)	12 (2.7)	
rs7041 (genotyped n = 904)	*CC*	366 (40.5)	166 (36.5)	200 (44.5)	0.040
	AC	440 (48.7)	233 (51.2)	207 (46.1)	
	AA	98 (10.8)	56 (12.3)	42 (9.4)	
rs1155563 (genotyped n = 840)	*TT*	523 (62.3)	242 (57.8)	281 (66.7)	0.047
	CT	279 (33.2)	155 (37.0)	124 (29.5)	
	CC	38 (4.5)	22 (5.3)	16 (5.3)	
Diplotype[b] (n = 886)	*1S/1S*	366 (41.3)	166 (37.6)	200 (45.0)	0.069
	1F/1S	180 (20.3)	91 (20.6)	89 (20.0)	
	1F/1F	16 (1.8)	6 (1.4)	10 (2.3)	
	1S/2	247 (27.9)	134 (30.3)	113 (25.5)	
	1F/2	43 (4.9)	22 (5)	21 (4.7)	
	2/2	34 (3.8)	23 (5.2)	11 (2.5)	
$Haplo_{3SNP}$[c] (n = 413)	*TGC*	364 (88.1)	164 (85.0)	200 (90.9)	0.035
	TGA	16 (3.9)	6 (3.1)	10 (4.5)	
	GTA	33 (8.0)	23 (11.9)	10 (4.5)	
$Haplo_{4SNP}$[d] (n = 355)	*TGCT*	323 (91.0)	145 (88.4)	178 (93.2)	0.062
	TGAT	11 (3.1)	4 (2.4)	7 (3.7)	
	GTAC	21 (5.9)	15 (9.1)	6 (3.1)	

[a]Pearson χ^2.

[b]rs4588/rs7041 diplotype.

[c]Haplotype of rs2282679, rs4588, and rs7041.

[d]Haplotype of all four studied SNPs.

increase of 25OHD in response to vitamin D supplementation in four adult studies (27, 44–46). Four studies have also found significant or indicative associations of major or minor alleles of rs7041 with, respectively, greater or smaller 25OHD increase in response to supplementation (25, 27, 45, 46). One study of pregnant women showed the rs2282679 major allele genotype to be associated with greater achieved 25OHD concentrations and changes thereof (26), and two adult studies have reported the minor allele genotype of rs2282679 to be related to smaller increase of 25OHD in response to vitamin D supplementation (25, 44).

Our study finds that in infants, major allele homozygotes of rs2282679, rs4588, and rs7041 as well as those homozygous for the major alleles of these three SNPs ($Haplo_{3SNP}$) show significantly greater supplementation responses to vitamin D_3 (30 μg/d) when compared with minor allele homozygotes. Vitamin D binding protein phenotype 1 is also linked to greater, and phenotype 2 to smaller, increases in 25OHD during intervention. DBP 1 phenotypes have been shown to correspond with higher 25OHD concentrations than DBP 2 phenotypes because DBP 1 has a higher affinity for 25OHD and is thought to prolong 25OHD half-life in plasma to a greater extent (17, 19). It is plausible that the observed differences in supplementation response are similarly explained by DBP phenotype–related effects on 25OHD concentrations and free and bioavailable 25OHD. The effects of genotype on supplementation response are seemingly dose dependent, with greater differences between variants seen at higher supplementation dosages.

Our results are in line with the majority of adult studies regarding differences in response to vitamin D supplementation between variants in the *GC* gene (25–27, 44–46). It has recently been suggested that genetic regulation of 25OHD concentration may be age dependent, with stronger associations reported in adults aged ≤60 compared with those >60 years, for some SNPs participating in vitamin D metabolism (47). It is possible that age-related differences in associations of genotype and response to supplementation could explain some of the differences in associations between our study and the minority of conflicting results found in older adult populations (28, 41–43).

The randomized, double-blinded intervention trial setting and the relatively large, homogenous study population, with uniform intervention and follow-up and in general very good compliance, are notable strengths of this study. Many previously reported studies of genotype-associated

Table 3. Differences in 25OHD Concentrations Between Variants During Follow-Up

		Baseline		12 mo				24 mo			
		All		Group 10		Group 30		Group 10		Group 30	
	Variant	$25OHD_{Adj}$[a] (nmol/L)	P_{Adj}[a]	$25OHD_{Adj}$[b] (nmol/L)	P_{Adj}[b]	$25OHD_{Adj}$[b] (nmol/L)	P_{Adj}[b]	$25OHD_{Adj}$[c] (nmol/L)	P_{Adj}[c]	$25OHD_{Adj}$[c] (nmol/L)	P_{Adj}[c]
rs2282679	*TT*	84.5 (82.2–86.8)	0.019	87.0 (84.3–89.8)	<0.001	119.9 (116.6–123.2)	<0.001	92.5 (89.9–95.1)	<0.001	123.6 (120.5–126.6)	<0.001
	GT	82.0 (78.8–85.1)		80.1 (76.6–83.6)		109.0 (104.2–113.9)		84.5 (81.2–87.8)		112.8 (108.3–117.2)	
	GG	72.3 (63.5–81.1)		69.6 (60.9–78.3)		88.0 (70.5–105.5)		70.7 (62.4–79.0)		102.2 (86.0–118.4)	
rs4588	*GG*	84.5 (82.2–86.8)	0.010	87.7 (85.0–90.5)	<0.001	120.0 (116.6–123.3)	<0.001	92.6 (89.9–95.2)	<0.001	123.6 (120.6–126.6)	<0.001
	GT	81.6 (78.4–84.7)		80.1 (76.7–83.6)		109.4 (104.5–114.3)		84.7 (81.5–88.0)		112.5 (108.1–117.0)	
	TT	71.5 (62.7–80.3)		68.1 (59.3–77.0)		92.7 (76.1–109.4)		70.8 (62.3–79.4)		104.5 (89.2–119.8)	
rs7041	*CC*	83.2 (80.4–86.0)	0.078	89.1 (85.7–92.6)	<0.001	118.9 (114.7–123.1)	0.124	92.7 (89.4–95.9)	<0.001	125.2 (121.5–129.0)	<0.001
	AC	84.3 (81.7–86.9)		82.1 (79.3–85.0)		114.8 (110.8–118.8)		88.0 (85.2–90.7)		116.4 (112.9–120.0)	
	AA	77.5 (72.2–82.9)		74.0 (68.3–79.7)		110.1 (101.6–118.6)		76.9 (71.2–82.5)		110.6 (102.9–118.4)	
rs1155563	*TT*	85.3 (82.9–87.7)	0.011	87.1 (84.2–90.0)	<0.001	119.3 (115.9–122.8)	<0.001	91.2 (88.5–94.0)	<0.001	123.0 (119.8–126.2)	<0.001
	CT	80.8 (77.5–84.0)		80.1 (76.6–83.7)		111.3 (106.2–116.4)		85.0 (81.7–88.3)		113.7 (109.1–118.3)	
	CC	74.7 (65.9–83.4)		69.7 (60.3–79.1)		95.4 (81.5–109.2)		70.5 (61.8–79.2)		102.0 (88.5–115.4)	
Diplotype[d]	*1S/1S*	83.1 (80.3–86.0)	0.028	89.2 (85.8–92.7)	<0.001	118.9 (114.8–123.0)	<0.001	92.9 (89.6–96.2)	<0.001	125.1 (121.4–128.9)	<0.001
	1F/1S	87.5 (83.5–91.6)		85.1 (80.5–89.8)		120.6 (114.7–126.5)		92.0 (87.6–96.5)		120.5 (115.1–126.0)	
	1F/1F	83.6 (69.9–97.4)		80.7 (63.4–97.9)		135.0 (118.5–151.5)		93.8 (77.2–110.4)		118.8 (103.5–134.1)	
	1S/2	81.8 (78.5–85.2)		80.5 (76.9–84.2)		109.8 (104.5–115.1)		85.6 (82.1–89.0)		113.2 (108.3–118.0)	
	1F/2	80.5 (72.5–88.5)		77.8 (68.7–86.9)		106.5 (94.8–118.2)		79.3 (70.5–88.1)		109.3 (98.1–120.5)	
	2/2	71.5 (62.7–80.3)		68.1 (59.3–76.9)		92.7 (76.2–109.2)		70.9 (62.3–79.4)		104.5 (89.1–119.9)	
$Haplo_{3SNP}$[e]	*TGC*	83.9 (81.5–86.3)	0.006	89.0 (85.2–92.7)	<0.001	117.7 (113.4–122.0)	0.001	93.5 (90.0–97.0)	<0.001	124.6 (120.5–128.6)	0.042
	TGA	83.6 (72.4–94.8)		81.2 (62.4–99.9)		134.4 (117.8–150.9)		96.4 (78.4–114.3)		118.6 (102.7–134.5)	
	GTA	71.4 (64.1–78.8)		67.7 (58.1–77.3)		87.0 (69.4–104.7)		69.6 (60.3–79.0)		102.8 (85.9–119.8)	
$Haplo_{4SNP}$[f]	*TGCT*	83.9 (81.4–86.4)	0.059	89.4 (85.5–93.4)	0.005	118.3 (113.7–123.0)	0.026	92.1 (88.4–95.7)	<0.001	124.7 (120.3–129.0)	0.065
	TGAT	84.5 (71.0–98.1)		86.5 (65.4–107.7)		127.1 (107.8–146.4)		100.7 (80.9–120.5)		125.3 (105.5–145.0)	
	GTAC	72.6 (63.5–81.6)		69.0 (57.5–80.4)		88.0 (64.8–111.3)		69.8 (59.2–80.3)		98.7 (77.2–120.2)	

Adjusted mean 25OHD concentrations by genotype, diplotype, and haplotype at baseline and at 12 and 24 mo of intervention and results for ANCOVA for differences between variants. Values are reported as adjusted mean serum 25OHD concentrations and 95% CI.

Significant differences in multiple comparisons (Bonferroni adjusted *P* values):

Baseline: rs2282679 and rs4588 major vs minor homozygotes ($P < 0.024$), Diplotype *2/2* vs *1F/1S* ($P = 0.017$), $Haplo_{3SNP}$ *TGC* vs *GTA* ($P = 0.004$).

12 mo: rs2282679, rs4588 and rs1155563 major homozygotes vs heterozygotes and minor homozygotes ($P < 0.040$) in both intervention groups, rs7041 all comparisons ($P < 0.035$) in Group10. Diplotype *1S/1S* vs *1S/2* and *2/2* ($P < 0.012$) and *1F/1S* vs *2/2* ($P = 0.012$) in Group 10, *2/2* vs *1S/1S*, *1F/1S*, *1F/1F* ($P < 0.038$) in Group 30. $Haplo_{3SNP}$ *TGC* vs *GTA* ($P < 0.001$) in Group 10, *TGC* vs *TGA* and *GTA* ($P < 0.004$) in Group 30, $Haplo_{4SNP}$ *TGCT* vs *GTAC* ($P = 0.003$) in Group 10, and *TGCT* vs *TGAT* and *GTAC* ($P < 0.037$) in Group 30.

24 months: rs2282679, rs4588 and rs1155563 all comparisons ($P < 0.011$) in Group 10, major homozygotes vs heterozygotes and minor homozygotes ($P < 0.050$) in Group30. Rs7041 *AA* vs *AC*, *CC* ($P < 0.002$) in Group 10 and *CC* vs *AC*, *AA* ($P < 0.004$) in Group 30. Diplotype *2/2* vs *1S/1S*, *1F/1S*, *1S/2* ($P < 0.029$) in Group10, *1S/1S* vs *1S/2* ($P = 0.001$) in Group 30. $Haplo_{3SNP}$ *TGC* vs *TGA* and *GTA* ($P < 0.030$) in Group 10, *TGC* vs *GTA* ($P = 0.043$) in Group 30, $Haplo_{4SNP}$ *TGCT* vs *TGAT* and *GTAC* ($P < 0.022$) in Group 10.

Number of subjects in analyses at baseline and at 12 and 24 mo, respectively:

Rs2282679 (n = 783/710/731), rs4588 (n = 789/716/733), rs7041 (n = 800/725/741), rs1155563 (n = 742/671/689), Diplotype (n = 784/710/728), $Haplo_{3SNP}$ (n = 366/319/338), $Haplo_{4SNP}$ (n = 317/285/294).

[a]Adjusted for season of birth, length-adjusted weight SDS at birth, and maternal vitamin D supplementation (μg/d) during pregnancy

[b]Adjusted for season of 12-mo follow-up, adherence to intervention supplement (0–12 mo) (%), length-adjusted weight SDS at 12 mo, and duration of breastfeeding up to 12 mo.

[c]Adjusted for season of 24-mo follow-up, adherence to intervention supplement (13–24 mo) (%), and length-adjusted weight SDS at 24 mo.

[d]rs4588/rs7041 diplotype.

[e]Haplotype of rs2282679, rs4588, and rs7041.

[f]Haplotype of all four studied SNPs.

Table 4. Mean Allelic Effects of Variants on 25OHD Concentrations During Follow-Up

Mean allelic effect size (nmol/L)	Baseline		12 mo				24 mo			
	All		Group 10		Group 30		Group 10		Group 30	
	B (95% CI)	P_{Adj}[a]	B (95% CI)	P_{Adj}[b]	B (95% CI)	P_{Adj}[b]	B (95% CI)	P_{Adj}[c]	B (95% CI)	P_{Adj}[c]
rs2282679 *T* > *G*	−3.8 (−6.9 to −0.8)	0.014	−7.9 (−11.2 to −4.5)	<0.001	−10.8 (−15.9 to −5.8)	<0.001	−9.0 (−12.3 to −5.8)	<0.001	−9.6 (−14.1 to −5.0)	<0.001
rs4588 *G* > *T*	−4.2 (−7.3 to −1.2)	0.006	−8.7 (−12.0 to −5.3)	<0.001	−9.9 (−14.9 to −4.9)	<0.001	−8.6 (−11.8 to −5.3)	<0.001	−9.6 (−14.0 to −5.1)	<0.001
rs7041 *C* > *A*	−1.7 (−4.3 to 0.9)	0.209	−7.6 (−10.6 to −4.6)	<0.001	−3.1 (−7.3 1.2)	0.154	−6.7 (−9.7 to −3.8)	<0.001	−6.9 (−10.6 to −3.1)	<0.001
rs1155563 *T* > *C*	−4.4 (−7.5 to −1.4)	0.005	−7.7 (−11.1 to −4.2)	<0.001	−8.5 (−13.5 to −3.6)	0.001	−8.0 (−11.3 to −4.6)	<0.001	−8.9 (−13.3 to −4.4)	<0.001
Diplotype[d] 1 > 2	−4.4 (−8.0 to −0.9)	0.015	−9.0 (−13.1 to −4.9)	<0.001	−10.3 (−16.0 to −4.6)	<0.001	−8.8 (−12.8 −4.8)	<0.001	−10.9 (−16.0 to −5.8)	<0.001
$Haplo_{3SNP}$[e] *TGC* > *GTA*	−13.8 (−23.1 to −4.5)	0.004	−21.4 (−31.0 to −11.9)	<0.001	−30.1 (−48.3 to −12.0)	0.001	−22.0 (−31.3 to −12.7)	<0.001	−16.1 (−32.9 to 0.6)	0.059
$Haplo_{4SNP}$[f] *TGCT* > *GTAC*	−12.6 (−24.1 to −1.1)	0.033	−22.5 (−34.2 to −10.8)	<0.001	−33.7 (−57.2 to −10.3)	0.005	−21.1 (−32.5 to −9.8)	<0.001	−18.3 (−39.9 to 3.3)	0.096

Adjusted mean allelic effects on 25OHD concentrations for the studied SNPs and adjusted mean effect size of diplotype and haplotype during intervention. Results for multivariate linear regression analyses. Values are reported as B coefficients and 95% CIs. Number of subjects in analyses: baseline: rs2282679 (n = 790), rs4588 (n = 796), rs7041 (n = 807), 1155563 (n = 748), diplotype (n = 791), $Haplo_{3SNP}$ (n = 356), $Haplo_{4SNP}$ (n = 310); 12 mo: rs2282679 (Group 10/Group 30; n = 382/387), rs4588 (n = 385/389), rs7041 (n = 393/391), rs1155563 (n = 361/366), diplotype (n = 382/387), $Haplo_{3SNP}$ (n = 157/186), $Haplo_{4SNP}$ (n = 135/165); 24 mo: rs2282679 (n = 365/372), rs4588 (n = 365/374), rs7041 (n = 371/376), 1155563 (n = 343/351), diplotype (n = 362/372), $Haplo_{3SNP}$ (n = 150/177), $Haplo_{4SNP}$ (n = 130/156).

[a] Adjusted for season of birth (spring vs other), length-adjusted weight SDS at birth, and maternal vitamin D supplementation (μg/d) during pregnancy.

[b] Adjusted for season of 12-mo follow-up (spring vs other), adherence to intervention supplement (0–12 mo) (%), length-adjusted weight SDS at 12 mo, and duration of breastfeeding up to 12 mo.

[c] Adjusted for season of 24-mo follow-up (spring vs other), adherence to intervention supplement (13–24 months) (%), and length-adjusted weight SDS at 24 mo.

[d] rs4588/rs7041 diplotype (1 = *1S/1S*, *1F/1S*, and *1F/1F* to 2 = *1S/2*, *1F/2*, and *2/2*).

[e] Haplotype of rs2282679, rs4588, and rs7041 (major to minor homozygotes, *TGC* > *GTA*).

[f] Haplotype of all four studied SNPs (major to minor homozygotes, *TGCT* > *GTAC*).

Table 5. Temporal Change of 25OHD Concentrations During Follow-Up in Group 30 by Genotype, Diplotype, and Haplotype

	Variant	$25OHD_{Adj}$ (nmol/L)			Repeated Measures ANCOVA	
		Baseline	12 mo	24 mo	$P_{variant}$[a]	$P_{interaction}$
rs2282679 (n = 367)	*TT*	82.5 (79.5–85.4)	120.2 (116.8–123.6)	123.2 (120.3–126.2)	<0.001	0.003
	GT	82.9 (78.5–87.2)	109.7 (104.6–114.7)	112.3 (107.8–116.7)		
	GG	76.1 (61.0–91.2)	86.4 (68.4–104.5)	102.2 (86.1–118.3)		
rs4588 (n = 369)	*GG*	82.4 (79.4–85.3)	120.2 (116.7–123.6)	123.3 (120.3–126.3)	<0.001	0.005
	GT	82.9 (78.6–87.3)	110.1 (105.0–115.2)	112.0 (107.6–116.4)		
	TT	74.4 (60.1–88.7)	91.8 (74.7–109.0)	104.0 (88.7–119.3)		
rs7041 (n = 371)	*CC*	82.0 (78.4–85.7)	119.2 (114.8–123.5)	125.0 (121.3–128.6)	0.013	0.018
	AC	83.5 (80.0–87.0)	115.4 (111.2–119.6)	115.9 (112.3–119.5)		
	AA	81.0 (73.4–88.7)	110.1 (101.1–119.1)	110.2 (102.4–118.0)		
rs1155563 (n = 346)	*TT*	84.0 (80.9–87.1)	119.8 (116.2–123.4)	122.8 (119.7–126.0)	<0.001	0.180
	CT	80.9 (76.3–85.5)	112.3 (107.0–117.6)	113.3 (108.6–117.9)		
	CC	77.3 (65.0–89.6)	95.7 (81.4–110.0)	101.3 (88.0–114.7)		
Diplotype[b] (n = 367)	*1S/1S*	81.9 (78.3–85.6)	119.0 (114.8–123.3)	124.9 (121.2–128.5)	<0.001	0.008
	1F/1S	83.7 (78.3–89.1)	120.7 (114.4,126.9)	120.0 (114.6,125.5)		
	1F/1F	84.0 (68.0,100.0)	138.1 (121.0–155.1)	118.3 (103.0–133.7)		
	1S/2	82.9 (78.1–87.6)	111.1 (105.6–116.5)	112.5 (107.6–117.3)		
	1F/2	83.2 (72.2–94.2)	104.6 (91.9–117.4)	109.3 (98.1–120.5)		
	2/2	74.4 (60.1–88.8)	91.7 (74.7–108.8)	104.0 (88.7–119.3)		
$Haplo_{3SNP}$[c] (n = 183)	*TGC*	81.6 (78.5–84.8)	118.6 (114.4–122.9)	124.5 (120.6–128.4)	0.001	0.011
	TGA	84.4 (71.0–97.7)	138.2 (121.2–155.2)	118.5 (102.6–134.3)		
	GTA	74.4 (61.7–87.2)	85.4 (67.3–103.5)	101.8 (85.1–118.6)		
$Haplo_{4SNP}$[d] (n = 159)	*TGCT*	82.0 (78.5–85.4)	119.6 (114.9–124.3)	124.8 (120.6–128.9)	0.005	0.314
	TGAT	88.2 (70.6–105.7)	126.8 (140.5–148.3)	124.6 (104.8–144.4)		
	GTAC	73.5 (57.4–89.7)	84.8 (61.4–108.2)	97.4 (76.1–118.6)		

Results of repeated measurement ANCOVA for differences in adjusted mean 25OHD concentrations ($P_{variant}$) and differences in temporal change between variants [i.e., interaction of variant and temporal change ($P_{interaction}$)]. Values are reported as adjusted mean serum 25OHD ($25OHD_{Adj}$) concentrations and 95% CIs. Means are adjusted for season of birth, length-adjusted weight SDS at 24 mo, adherence to intervention supplementation (%) throughout the intervention (0–24 mo), as well as interaction of adherence to supplementation and temporal change.

[a]Significant differences in multiple comparisons (Bonferroni adjusted *P* values): rs2282679: *TT* vs *GT* and *GG* ($P = 0.001$); rs4588: *GG* vs *GT* and *TT* ($P < 0.003$); rs7041: *CC* vs *AC* ($P = 0.024$); rs1155563: *TT* vs *CT* and *CC* ($P < 0.004$); diplotype: *2/2* vs *1S/1S*, *1S/1F*, and *1F/1F* ($P < 0.037$); $Haplo_{3SNP}$: *GTA* vs *TGC* and *TGA* ($P < 0.003$); $Haplo_{4SNP}$: *GTAC* vs *TGCT* and *TGAT* ($P < 0.019$).

[b]rs4588/rs7041 diplotype.

[c]Haplotype of rs2282679, rs4588, and rs7041.

[d]Haplotype of all four studied SNPs.

differences in vitamin D supplementation response have been performed in smaller study populations and/or by pooling data from several different trials. Although our study included 913 infants, some genotypes, and consequently diplotypes and haplotypes, are quite rare, and the number of subjects was a limitation in this study. To increase power, combinations of haplotypes of the three SNPs most consistently associated with 25OHD concentrations and temporal changes thereof (rs2282679, rs4588, and rs7041) were used.

We recognize some limitations in our study setting. It was not possible to obtain data on nutritional vitamin D intake, including more detailed information on total amount and vitamin D contents of breast milk, for the entire 24-month follow-up period. Due to the randomized study setting, nutritional vitamin D intake did not differ between our intervention groups at 12 months of age (30). Data on DBP concentrations were not available for this study. The *GC* genotype has been reported to affect 25OHD concentrations through both quantitative differences of DBP and genotype-associated functional differences of the binding protein (48). Supplementation dose has, however, previously been reported not to affect DBP concentration (18).

Optimal vitamin D supplementation and 25OHD concentrations in infants are still under discussion, with some international guidelines currently recommending higher doses of up to 25 μg/d for children >1 year of age (1, 2, 49, 50). Although genotype does not seem to affect response to current 10 μg/d supplementation, the observed differences between genotype-defined "poor" and "good" responders are important at higher supplementation doses and should be considered when evaluating changes to supplementation guidelines in the studied age group. Whether our findings could improve tailoring individual treatment of vitamin D deficiency, where

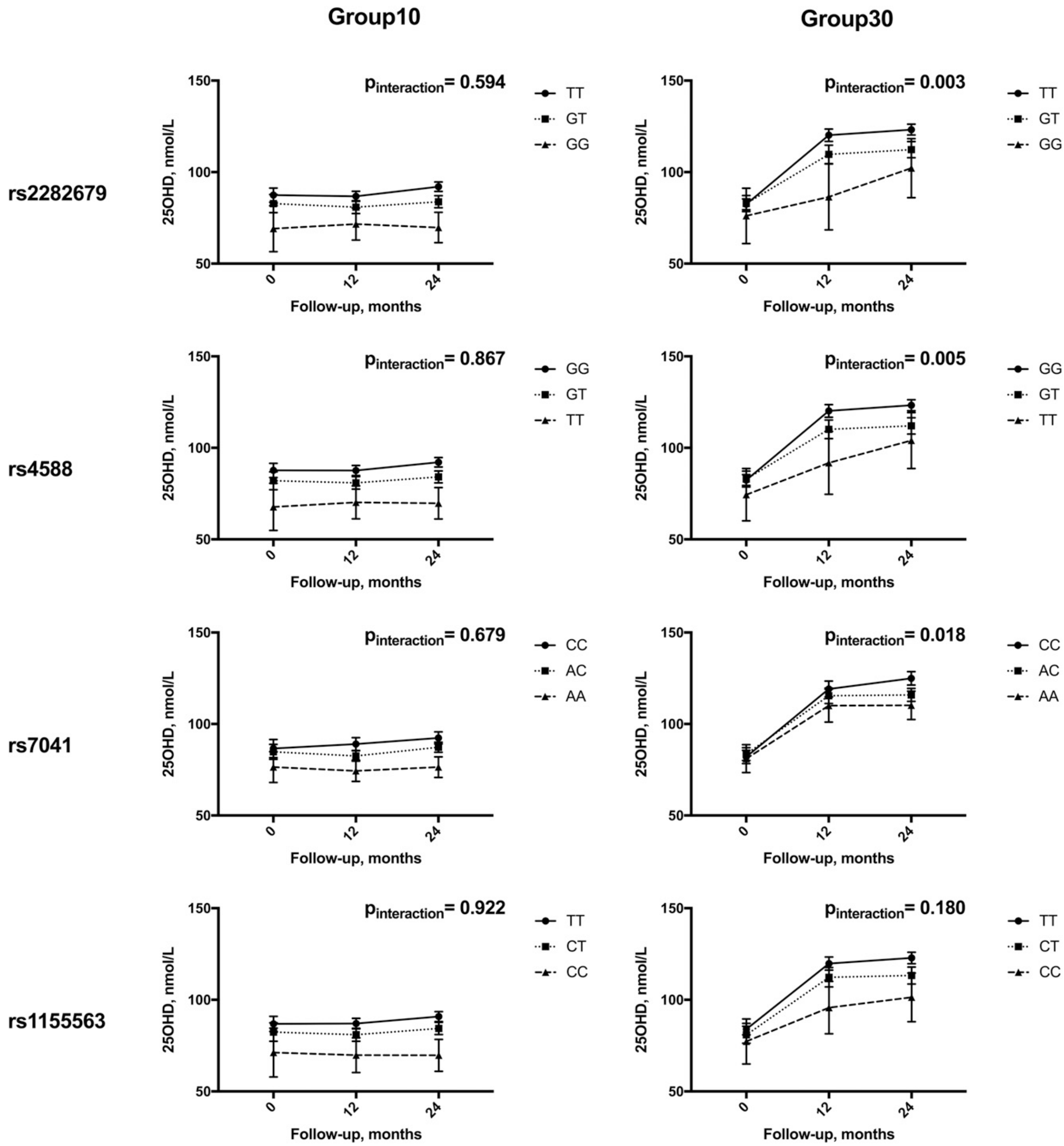

Figure 1. Temporal change of mean serum 25OHD concentrations (nmol/L) during follow-up by genotype in the two intervention groups (Group 10 and Group 30), with results of interaction between variants and temporal change during followup in repeated measures ANCOVA ($P_{\text{interaction}}$). In Group 10, means are adjusted for season of birth, length-adjusted weight SDS at 24 mo, duration of breastfeeding (mo), adherence to intervention supplementation (%) throughout the intervention (0 to 24 mo), and interaction of adherence to supplementation and temporal change. In Group 30, means are adjusted for season of birth, length-adjusted weight SDS at 24 mo, adherence to intervention supplementation (%) throughout the intervention (0 to 24 mo), and interaction of adherence to supplementation and temporal change (breastfeeding was not a significant covariant in this group).

notably greater vitamin D doses are used, requires further studies.

In line with recent findings in Finnish adults, showing a clear decrease in vitamin D deficiency after increased fortification and supplementation guidelines in Finland (4, 51), our study population was mainly vitamin D sufficient at all time points in both intervention groups. It is therefore difficult to draw conclusions on the consequences of our findings, or potential genotype-associated differences in response to current supplementation, in vitamin D–deficient populations. In light of the observed differences in vitamin D supplementation

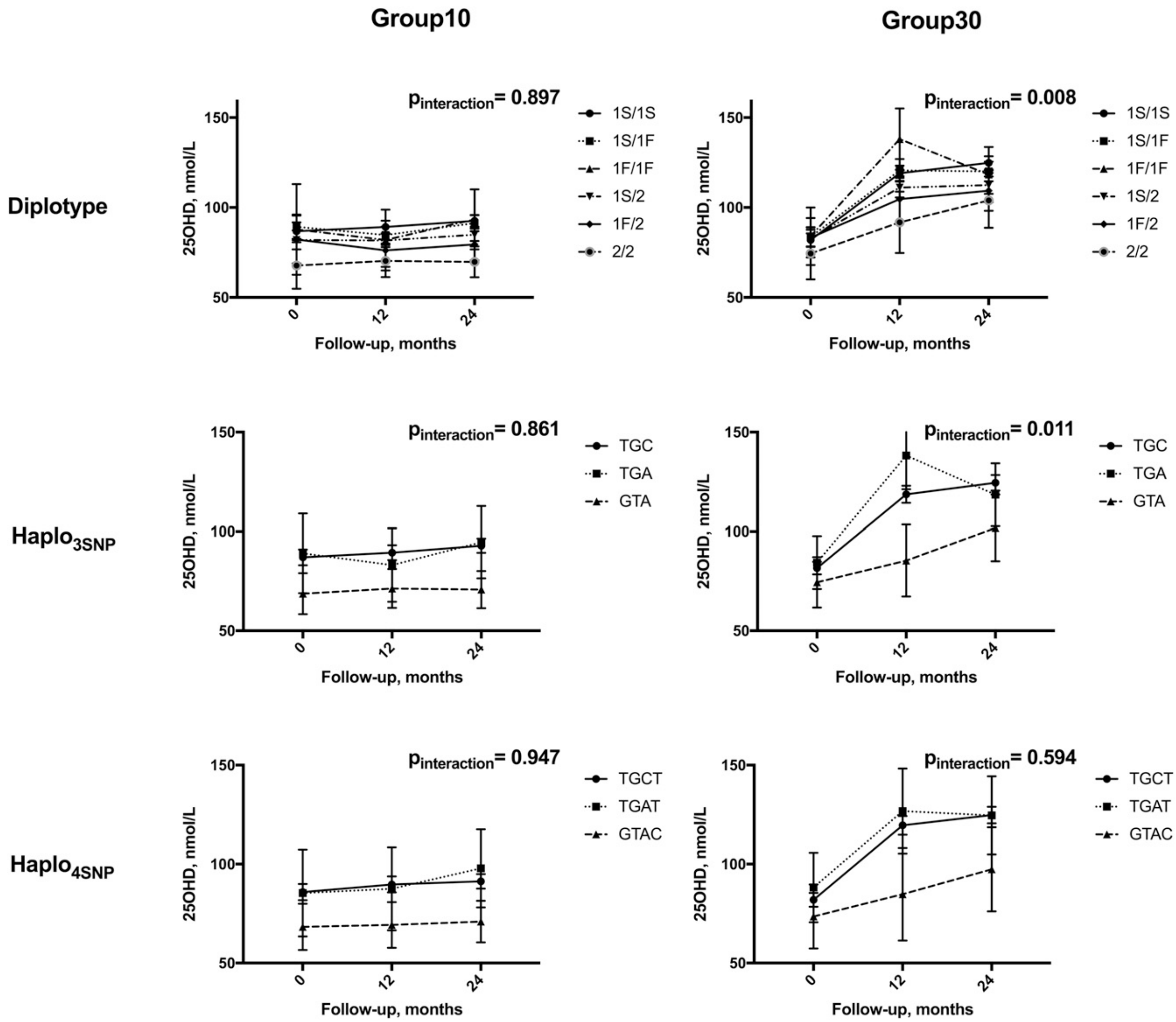

Figure 2. Temporal change of mean serum 25OHD concentrations (nmol/L) during follow-up by diplotypes and haplotypes in the two intervention groups (Group 10 and Group 30), with results of interaction between variants and temporal change during follow-up in repeated measures ANCOVA ($P_{\text{interaction}}$). In Group 10, means are adjusted for season of birth, length-adjusted weight SDS at 24 mo, duration of breastfeeding (mo), adherence to intervention supplementation (%) throughout the intervention (0 to 24 mo), and interaction of adherence to supplementation and temporal change. In Group 30, means are adjusted for season of birth, length-adjusted weight SDS at 24 months, adherence to intervention supplementation (%) throughout the intervention (0 to 24 mo), and interaction of adherence to supplementation and temporal change (breastfeeding was not a significant covariant in this group).

response, it seems feasible that vitamin D–deficient minor allele homozygotes of the studied *GC* variants could require higher supplementation doses to achieve optimal 25OHD concentrations and to avoid the skeletal and extraskeletal effects of vitamin D deficiency. This should, however, be evaluated by separate prospective studies in which intervention participants are stratified by genotype of the vitamin D binding protein.

We have previously reported that there was no significant difference in parent-reported infections or in bone strength between the two intervention groups of the VIDI trial (30). Genotype of *GC* variants have been associated with differences in bone strength (rs4588) (19) and extraskeletal effects, including effects on inflammation and immunity (rs4588/rs7041 diplotype) (52). Whether *GC* genotype–related differences in supplementation response translates into differences in vitamin D–dependent outcomes, such as bone strength and inflammation, warrants further studies, possibly with a wider spectrum of variants of the *GC* gene.

In summary, our study involving infants from birth to 24 months found that, in addition to associations between *GC* SNPs and 25OHD, the haplotypes of rs2282679, rs4588, rs7041, and rs1155563 significantly affected 25OHD concentrations. Genotype of rs2282679, rs4588, and rs7041; their haplotype; and rs4588/rs7041 diplotype also significantly modified response to 24-month high-dose supplementation of 30 μg/d vitamin D_3. Genetic predisposition in the *GC*

Table 6. Mean Change of Serum Δ25OHD by Genotype, Diplotype, and Haplotype From Baseline to 24 mo of Intervention in Participants With >80% Adherence to Intervention Vitamin D_3 Supplementation

		All (Adherence >80%)			Group 10 (Adherence >80%)			Group 30 (Adherence >80%)		
	Variant	Δ25OHD (nmol/L)	$P^{\dagger}$	$P_{adj}^{a\dagger\dagger}$	Δ25OHD (nmol/L)	P	P_{adj}^{b}	Δ25OHD (nmol/L)	$P^{\dagger\dagger\dagger}$	$P_{adj}^{b\dagger\dagger\dagger\dagger}$
rs2282679 (n = 603)	*TT*	25.3 (38.7)	0.001	0.016	5.5 (36.5)	0.730	0.673	43.2 (31.3)	0.006	0.004
	GT	15.0 (35.1)			2.4 (30.0)			30.6 (34.8)		
	GG	8.2 (19.7)			2.6 (18.8)			25.7 (10.3)		
rs4588 (n = 609)	*GG*	25.3 (38.8)	0.003	0.021	5.5 (36.6)	0.859	0.771	43.2 (31.3)	0.005	0.004
	GT	15.2 (34.8)			3.4 (29.9)			30.2 (34.9)		
	TT	11.6 (21.7)			4.0 (18.3)			31.2 (17.5)		
rs7041 (n = 614)	*CC*	27.9 (35.4)	0.001	0.010	6.6 (29.8)	0.731	0.814	45.6 (29.4)	0.002	0.002
	AC	16.9 (38.1)			3.7 (37.0)			33.5 (32.8)		
	AA	14.1 (34.1)			2.9 (24.5)			29.2 (39.5)		
rs1155563 (n = 566)	*TT*	24.2 (39.4)	0.011	0.182	4.7 (37.1)	0.891	0.899	41.2 (33.0)	0.068	0.073
	CT	15.9 (34.4)			3.9 (30.0)			33.0 (33.1)		
	CC	8.3 (24.3)			0.6 (20.6)			22.8 (25.2)		
Diplotype[c] (n = 604)	*1S/1S*	27.9 (35.4)	0.004	0.035	6.6 (29.8)	0.682	0.631	45.6 (29.4)	0.022	0.016
	1F/1S	18.7 (45.8)			2.5 (47.3)			36.9 (36.5)		
	1F/1F	31.8 (22.3)			24.0 (31.2)			36.3 (16.6)		
	1S/2	16.1 (32.8)			4.4 (29.9)			31.6 (30.2)		
	1F/2	10.2 (43.9)			−4.6 (26.4)			25.0 (53.1)		
	2/2	11.6 (21.7)			4.0 (18.3)			31.2 (17.5)		
$Haplo_{3SNP}$[d] (n = 282)	*TGC*	27.9 (35.4)	0.035	0.401	6.6 (29.8)	0.448	0.304	45.6 (29.4)	0.188	0.253
	TGA	31.8 (22.3)			24.0 (31.2)			36.3 (16.6)		
	GTA	9.4 (19.1)			4.0 (18.3)			25.7 (10.3)		
$Haplo_{4SNP}$[e] (n = 244)	*TGCT*	27.7 (35.8)	0.076	0.449	5.3 (29.3)	0.129	0.082	45.8 (29.8)	0.354	0.468
	TGAT	39.2 (14.7)			39.1 (9.2)			39.3 (18.3)		
	GTAC	10.0 (19.8)			5.3 (20.2)			25.4 (6.2)		

Results are for ANOVA and ANCOVA for differences between variants. Values are reported as means and SD.

[a]Adjusted for season of birth, adherence to intervention supplement (0–24 mo) (%), length-adjusted weight SDS at 24 mo, and intervention group.

[b]Adjusted for season of birth, adherence to intervention supplement (0–24 mo) (%) and length-adjusted weight SDS at 24 mo

Significant differences in multiple comparisons:

[†]rs2282679: *TT* vs *GT* and *GG* (Tamhane $P < 0.005$), rs4588: *GG* vs *GT* and *TT* (Tamhane $P < 0.022$), rs7041: *CC* vs *AC* and *AA* (Bonferroni $P < 0.020$), rs1155563: *TT* vs *CT* and *CC* (Tamhane $P < 0.033$), Diplotype: *1S/1S* vs *1S/2* and *2/2* (Tamhane $P < 0.029$), $Haplo_{3SNP}$: *GTA* vs *TGC* and *TGA* (Tamhane $P < 0.032$); $Haplo_{4SNP}$: *GTAC* vs *TGCT* and *TGAT* (Tamhane $P < 0.010$; Welch test of equality of means $P = 0.002$).

[††]rs2282679: *TT* vs *GT* (Bonferroni $P = 0.016$), rs4588: *GG* vs *GT* (Bonferroni $P = 0.018$), rs7041: *CC* vs *AC* (Bonferroni $P = 0.020$).

[†††]rs2282679: *TT* vs *GT* (Bonferroni $P = 0.007$), rs4588: *GG* vs *GT* (Bonferroni $P = 0.005$), rs7041: *CC* vs *AC* and *AA* (Bonferroni $P < 0.040$), Diplotype: *1S/1S* vs *1S/2* (Bonferroni $P = 0.034$), $Haplo_{3SNP}$: *GTA* vs *TGC* (Tamhane $P = 0.008$, Welch test of equality of means $P = 0.008$).

[††††]rs2282679: *TT* vs *GT* (Bonferroni $P = 0.004$), rs4588: *GG* vs *GT* (Bonferroni $P = 0.003$), rs7041: *CC* vs *AC* and *AA* (Bonferroni $P < 0.037$), Diplotype: *1S/1S* vs *1S/2* (Bonferroni $P = 0.021$).

[c]rs4588/rs7041 diplotype.

[d]Haplotype of rs2282679, rs4588, and rs7041.

[e]Haplotype of all four studied SNPs.

and other genes of vitamin D metabolism may have a notable impact on individual 25OHD concentrations and response to vitamin D supplementation. Further studies are warranted for a more complete understanding of the effects of genetic variation of the vitamin D binding protein on the response to supplementation and consequences thereof as well as possible identification of those in need of greater supplementation doses.

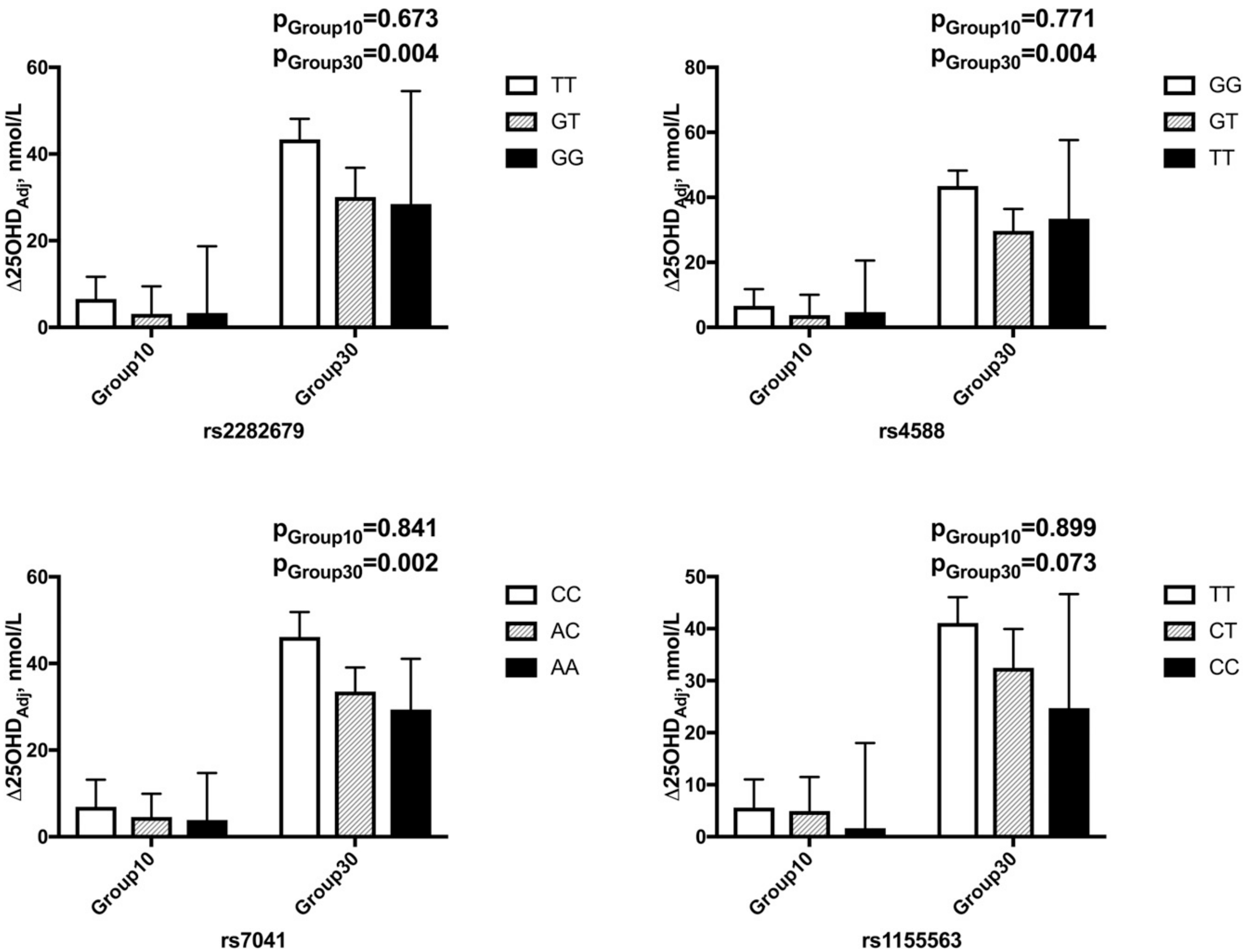

Figure 3. Adjusted mean change of serum 25OHD concentration ($\Delta 25OHD_{Adj}$) (nmol/L) in the two intervention groups (Group 10 and Group 30) by genotype, diplotype, and haplotype from baseline to 24 mo of intervention in participants with >80% adherence to intervention vitamin D_3 supplementation. Results for ANCOVAs for differences between variants. Means are adjusted for season of birth, adherence to intervention supplementation (%) throughout the intervention (0 to 24 mo), and length-adjusted weight SDS at 24 mo.

Acknowledgments

The authors thank the personnel of the Kätilöopisto Maternity Hospital in Helsinki and the Folkhälsan Research Center and our study nurses Sirpa Nolvi, Rhea Paajanen, Päivi Turunen, Nea Boman, and Sari Lindén for their contributions. They also wish to express their gratitude to the participating families.

Financial Support: The research for this study was supported by grants from the Finnish Medical Foundation, Victoriastiftelsen, the Orion Research Foundation, the Instrumentarium Science Foundation, and the Paulo Foundation (all to M.E.C.); the Päivikki and Sakari Sohlberg Foundation and the Juho Vainio Foundation (both to H.H.); the Finnish Pediatric Research Foundation, the Academy of Finland, the Sigrid Jusélius Foundation, the Swedish Research Council, the Novo Nordisk Foundation, the Swedish Childhood Cancer Foundation, and the Folkhälsan Research Foundation (all to O.M.); and Finska Läkaresällskapet, Stiftelsen Dorothea Olivia, Karl Walter och Jarl Walter Perkléns Minne, and state funding for university-level health research in Finland (all to S.A.).

Clinical Trial Information: ClinicalTrials.gov no. NCT01723852 (registered 6 November 2012).

Author Contributions: M.E.-C., S.A., O.M., and M.P. designed the study. M.E.-C., L.K., S.A., O.M., and M.P. conducted the research. M.E.-C. and M.P. analyzed the data. M.E.-C. wrote the first draft of the manuscript. M.E.-C., L.K., E.H.-S., H.H.A., J.R., S.V., O.H., T.H., H.V., S.A., O.M., and M.P. took wrote and edited the manuscript. M.E.-C., S.A., O.M., and M.P. have primary responsibility for the final content. All authors read and approved the final version of the manuscript.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

References and Notes

1. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;**357**(3): 266–281.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;**96**(7):1911–1930.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab*. 2012;**97**(4):1153–1158.
4. Raulio S, Erlund I, Männistö S, Sarlio-Lähteenkorva S, Sundvall J, Tapanainen H, Vartiainen E, Virtanen SM. Successful nutrition policy: improvement of vitamin D intake and status in Finnish adults over the last decade. *Eur J Public Health*. 2017;**27**(2): 268–273.
5. Holick MF. Vitamin D: extraskeletal health. *Endocrinol. Metab. Clin. North Am*. 2010;**39**(2):381–400.
6. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Mäkitie O, Ramos-Abad L, Ward L, DiMeglio LA, Atapattu N, Cassinelli H, Braegger C, Pettifor JM, Seth A, Idris HW, Bhatia V, Fu J, Goldberg G, Sävendahl L, Khadgawat R, Pludowski P, Maddock J, Hyppönen E, Oduwole A, Frew E, Aguiar M, Tulchinsky T, Butler G, Högler W. Global consensus recommendations on prevention and management of nutritional rickets. *J Clin Endocrinol Metab*. 2016; **101**(2):394–415.
7. Bouillon R. Extra-skeletal effects of vitamin D. *Front Horm Res*. 2018;**50**:72–88.
8. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung C-L, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen A-L, Zhai G, Macdonald HM, Forouhi NG, Loos RJF, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson J-O, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin M-R, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. 2010;**376**(9736): 180–188.
9. Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. *Clin Chem*. 2001;**47**(4):753–756.
10. McGrath JJ, Saha S, Burne THJ, Eyles DW. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. *J Steroid Biochem Mol Biol*. 2010;**121**(1-2):471–477.
11. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, Li Q, Weinstein SJ, Purdue M, Virtamo J, Horst R, Wheeler W, Chanock S, Hunter DJ, Hayes RB, Kraft P, Albanes D. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet*. 2010;**19**(13):2739–2745.
12. Gozdzik A, Zhu J, Wong BYL, Fu L, Cole DEC, Parra EJ. Association of vitamin D binding protein (VDBP) polymorphisms and serum 25(OH)D concentrations in a sample of young Canadian adults of different ancestry. *J Steroid Biochem Mol Biol*. 2011; **127**(3-5):405–412.
13. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, Bowden DW, Norris JM. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab*. 2008;**93**(9):3381–3388.
14. Speeckaert M, Huang G, Delanghe JR, Taes YEC. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. *Clin Chim Acta*. 2006;**372**(1-2):33–42.
15. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *J Clin Endocrinol Metab*. 1986;**63**(4):954–959.
16. Cleve H, Constans J. The mutants of the vitamin-D-binding protein: more than 120 variants of the GC/DBP system. *Vox Sang*. 1988;**54**(4):215–225.
17. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, Nexo E. Plasma concentrations of 25-hydroxyvitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int*. 2005; **77**(1):15–22.
18. Sollid ST, Hutchinson MYS, Berg V, Fuskevåg OM, Figenschau Y, Thorsby PM, Jorde R. Effects of vitamin D binding protein phenotypes and vitamin D supplementation on serum total 25(OH)D and directly measured free 25(OH)D. *Eur J Endocrinol*. 2016; **174**(4):445–452.
19. Pekkinen M, Saarnio E, Viljakainen HT, Kokkonen E, Jakobsen J, Cashman K, Mäkitie O, Lamberg-Allardt C. Vitamin D binding protein genotype is associated with serum 25-hydroxyvitamin D and PTH concentrations, as well as bone health in children and adolescents in Finland. *PLoS ONE* 2014;**9**(1):e87292.
20. Batai K, Murphy AB, Shah E, Ruden M, Newsome J, Agate S, Dixon MA, Chen HY, Deane LA, Hollowell CMP, Ahaghotu C, Kittles RA. Common vitamin D pathway gene variants reveal contrasting effects on serum vitamin D levels in African Americans and European Americans. *Hum Genet*. 2014;**133**(11):1395–1405.
21. Nissen J, Rasmussen LB, Ravn-Haren G, Andersen EW, Hansen B, Andersen R, Mejborn H, Madsen KH, Vogel U. Common variants in CYP2R1 and GC genes predict vitamin D concentrations in healthy Danish children and adults. *PLoS ONE* 2014;**9**(2):e89907.
22. Anderson D, Holt BJ, Pennell CE, Holt PG, Hart PH, Blackwell JM. Genome-wide association study of vitamin D levels in children: replication in the Western Australian Pregnancy Cohort (Raine) study. *Genes Immun*. 2014;**15**(8):578–583.
23. Størdal K, Mårild K, Tapia G, Haugen M, Cohen AS, Lie BA, Stene LC. Fetal and maternal genetic variants influencing neonatal vitamin D status. *J Clin Endocrinol Metab*. 2017;**102**(11): 4072–4079.
24. Sollid ST, Hutchinson MYS, Fuskevåg OM, Joakimsen RM, Jorde R. Large Individual differences in serum 25-hydroxyvitamin D response to vitamin D supplementation: effects of genetic factors, body mass index, and baseline concentration. Results from a Randomized Controlled Trial. *Horm Metab Res*. 2016;**48**(1): 27–34.
25. Didriksen A, Grimnes G, Hutchinson MS, Kjærgaard M, Svartberg J, Joakimsen RM, Jorde R. The serum 25-hydroxyvitamin D response to vitamin D supplementation is related to genetic factors, BMI, and baseline levels. *Eur J Endocrinol*. 2013;**169**(5):559–567.
26. Moon RJ, Harvey NC, Cooper C, D'Angelo S, Curtis EM, Crozier SR, Barton SJ, Robinson SM, Godfrey KM, Graham NJ, Holloway JW, Bishop NJ, Kennedy S, Papageorghiou AT, Schoenmakers I,

Fraser R, Gandhi SV, Prentice A, Inskip HM, Javaid MK; Maternal Vitamin D Osteoporosis Study Trial Group. Response to antenatal cholecalciferol supplementation is associated with common vitamin D-related genetic variants. *J Clin Endocrinol Metab*. 2017; **102**(8):2941–2949.
27. Nimitphong H, Saetung S, Chanprasertyotin S, Chailurkit L-O, Ongphiphadhanakul B. Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D_3 or D_2 supplementation. *Nutr J*. 2013;**12**(1):39.
28. Fu L, Yun F, Oczak M, Wong BYL, Vieth R, Cole DEC. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. *Clin Biochem*. 2009;**42**(10-11): 1174–1177.
29. Helve O, Viljakainen H, Holmlund-Suila E, Rosendahl J, Hauta-Alus H, Enlund-Cerullo M, Valkama S, Heinonen K, Räikkönen K, Hytinantti T, Mäkitie O, Andersson S. Towards evidence-based vitamin D supplementation in infants: vitamin D intervention in infants (VIDI) - study design and methods of a randomised controlled double-blinded intervention study. *BMC Pediatr*. 2017; **17**(1):91.
30. Rosendahl J, Valkama S, Holmlund-Suila E, Enlund-Cerullo M, Hauta-Alus H, Helve O, Hytinantti T, Levälahti E, Kajantie E, Viljakainen H, Mäkitie O, Andersson S. Effect of higher vs standard dosage of vitamin D3 supplementation on bone strength and infection in healthy infants. *JAMA Pediatr*. 2018;**172**(7):646–654.
31. Ministerråd N. *Nordic Nutrition Recommendations*: Integrating Nutrition and Physical Activity. Copenhagen, Denmark: Nordic Council of Ministers; 2014.
32. Saari A, Sankilampi U, Hannila M-L, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med*. 2010; **43**(3):235–248.
33. International HapMap Consortium. A haplotype map of the human genome. *Nature*. 2005;**437**(7063):1299–1320.
34. Saarnio E, Pekkinen M, Itkonen ST, Kemi V, Karp H, Kärkkäinen M, Mäkitie O, Lamberg-Allardt C. Serum parathyroid hormone is related to genetic variation in vitamin D binding protein with respect to total, free, and bioavailable 25-hydroxyvitamin D in middle-aged Caucasians: a cross-sectional study. *BMC Nutr*. 2016; **2**(1):46.
35. Braun A, Bichlmaier R, Cleve H. Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types. *Hum Genet*. 1992;**89**(4):401–406.
36. Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J, Billis K, Cummins C, Gall A, Girón CG, Gil L, Gordon L, Haggerty L, Haskell E, Hourlier T, Izuogu OG, Janacek SH, Juettemann T, To JK, Laird MR, Lavidas I, Liu Z, Loveland JE, Maurel T, McLaren W, Moore B, Mudge J, Murphy DN, Newman V, Nuhn M, Ogeh D, Ong CK, Parker A, Patricio M, Riat HS, Schuilenburg H, Sheppard D, Sparrow H, Taylor K, Thormann A, Vullo A, Walts B, Zadissa A, Frankish A, Hunt SE, Kostadima M, Langridge N, Martin FJ, Muffato M, Perry E, Ruffier M, Staines DM, Trevanion SJ, Aken BL, Cunningham F, Yates A, Flicek P. Ensembl 2018. *Nucleic Acids Res*. 2017;**46**(D1):D754–D761.
37. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won H-H, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**(7616):285–291.
38. Sequencing Initiative Suomi project (SISu). SISu v4.1, October, 2018. Available at: www.sisuproject.fi. Accessed 13 September 2018.
39. Suaini NHA, Koplin JJ, Ellis JA, Peters RL, Ponsonby A-L, Dharmage SC, Matheson MC, Wake M, Panjari M, Tan H-TT, Martin PE, Pezic A, Lowe AJ, Martino D, Gurrin LC, Vuillermin PJ, Tang MLK, Allen KJ, HealthNuts Study Investigators. Environmental and genetic determinants of vitamin D insufficiency in 12-month-old infants. J. Steroid Biochem. *Mol. Biol*. 2014;**144**(Pt B):445–454.
40. Carpenter TO, Zhang JH, Parra E, Ellis BK, Simpson C, Lee WM, Balko J, Fu L, Wong BYL, Cole DEC. Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers. *J Bone Miner Res*. 2012;**28**(1):213–221.
41. Barry EL, Rees JR, Peacock JL, Mott LA, Amos CI, Bostick RM, Figueiredo JC, Ahnen DJ, Bresalier RS, Burke CA, Baron JA. Genetic variants in CYP2R1, CYP24A1, and VDR modify the efficacy of vitamin D3 supplementation for increasing serum 25-hydroxyvitamin D levels in a randomized controlled trial. *J Clin Endocrinol Metab*. 2014;**99**(10):E2133–E2137.
42. Waterhouse M, Tran B, Armstrong BK, Baxter C, Ebeling PR, English DR, Gebski V, Hill C, Kimlin MG, Lucas RM, Venn A, Webb PM, Whiteman DC, Neale RE. Environmental, personal, and genetic determinants of response to vitamin D supplementation in older adults. *J Clin Endocrinol Metab*. 2014;**99**(7): E1332–E1340.
43. Zhang M, Zhao L-J, Zhou Y, Badr R, Watson P, Ye A, Zhou B, Zhang J, Deng H-W, Recker RR, Lappe JM. SNP rs11185644 of RXRA gene is identified for dose-response variability to vitamin D3 supplementation: a randomized clinical trial. *Sci Rep*. 2017;**7**(1): 40593.
44. Nissen J, Vogel U, Ravn-Haren G, Andersen EW, Madsen KH, Nexø BA, Andersen R, Mejborn H, Bjerrum PJ, Rasmussen LB, Wulf HC. Common variants in CYP2R1 and GC genes are both determinants of serum 25-hydroxyvitamin D concentrations after UVB irradiation and after consumption of vitamin D_3-fortified bread and milk during winter in Denmark. *Am J Clin Nutr*. 2014; **101**(1):218–227.
45. Gaffney-Stomberg E, Lutz LJ, Shcherbina A, Ricke DO, Petrovick M, Cropper TL, Cable SJ, McClung JP. Association between single gene polymorphisms and bone biomarkers and response to calcium and vitamin D supplementation in young adults undergoing military training. *J Bone Miner Res*. 2016; **32**(3):498–507.
46. Yao P, Sun L, Lu L, Ding H, Chen X, Tang L, Xu X, Liu G, Hu Y, Ma Y, Wang F, Jin Q, Zheng H, Yin H, Zeng R, Chen Y, Hu FB, Li H, Lin X. Effects of genetic and nongenetic factors on total and bioavailable 25(OH)D responses to vitamin D supplementation. *J Clin Endocrinol Metab*. 2017;**102**(1):100–110.
47. Miettinen ME, Smart MC, Kinnunen L, Keinänen-Kiukaanniemi S, Moilanen L, Puolijoki H, Saltevo J, Oksa H, Hitman GA, Tuomilehto J, Peltonen M. The effect of age and gender on the genetic regulation of serum 25-hydroxyvitamin D: the FIN-D2D population-based study. *J Steroid Biochem Mol Biol*. 2018;**178**: 229–233.
48. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med*. 2013; **369**(21):1991–2000.
49. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord*. 2017;**18**(2):153–165.

50. Pludowski P, Holick MF, Grant WB, Konstantynowicz J, Mascarenhas MR, Haq A, Povoroznyuk V, Balatska N, Barbosa AP, Karonova T, Rudenka E, Misiorowski W, Zakharova I, Rudenka A, Łukaszkiewicz J, Marcinowska-Suchowierska E, Łaszcz N, Abramowicz P, Bhattoa HP, Wimalawansa SJ. Vitamin D supplementation guidelines. *J Steroid Biochem Mol Biol*. 2018; **175**:125–135.
51. Jääskeläinen T, Itkonen ST, Lundqvist A, Erkkola M, Koskela T, Lakkala K, Dowling KG, Hull GL, Kröger H, Karppinen J, Kyllönen E, Härkänen T, Cashman KD, Männistö S, Lamberg-Allardt C. The positive impact of general vitamin D food fortification policy on vitamin D status in a representative adult Finnish population: evidence from an 11-y follow-up based on standardized 25-hydroxyvitamin D data. *Am J Clin Nutr*. 2017;**105**(6): 1512–1520.
52. Malik S, Fu L, Juras DJ, Karmali M, Wong BYL, Gozdzik A, Cole DEC. Common variants of the vitamin D binding protein gene and adverse health outcomes. *Crit Rev Clin Lab Sci*. 2013;**50**(1):1–22.