

Common genetic determinants of vitamin D insufficiency: a genome-wide association study



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Summary

Background Vitamin D is crucial for maintenance of musculoskeletal health, and might also have a role in extraskeletal tissues. Determinants of circulating 25-hydroxyvitamin D concentrations include sun exposure and diet, but high heritability suggests that genetic factors could also play a part. We aimed to identify common genetic variants affecting vitamin D concentrations and risk of insufficiency.

Methods We undertook a genome-wide association study of 25-hydroxyvitamin D concentrations in 33 996 individuals of European descent from 15 cohorts. Five epidemiological cohorts were designated as discovery cohorts (n=16 125), five as in-silico replication cohorts (n=9367), and five as de-novo replication cohorts (n=8504). 25-hydroxyvitamin D concentrations were measured by radioimmunoassay, chemiluminescent assay, ELISA, or mass spectrometry. Vitamin D insufficiency was defined as concentrations lower than 75 nmol/L or 50 nmol/L. We combined results of genome-wide analyses across cohorts using Z-score-weighted meta-analysis. Genotype scores were constructed for confirmed variants.

Findings Variants at three loci reached genome-wide significance in discovery cohorts for association with 25-hydroxyvitamin D concentrations, and were confirmed in replication cohorts: 4p12 (overall $p=1.9 \times 10^{-109}$ for rs2282679, in GC); 11q12 ($p=2.1 \times 10^{-27}$ for rs12785878, near *DHCR7*); and 11p15 ($p=3.3 \times 10^{-20}$ for rs10741657, near *CYP2R1*). Variants at an additional locus (20q13, *CYP24A1*) were genome-wide significant in the pooled sample ($p=6.0 \times 10^{-10}$ for rs6013897). Participants with a genotype score (combining the three confirmed variants) in the highest quartile were at increased risk of having 25-hydroxyvitamin D concentrations lower than 75 nmol/L (OR 2.47, 95% CI 2.20–2.78, $p=2.3 \times 10^{-48}$) or lower than 50 nmol/L (1.92, 1.70–2.16, $p=1.0 \times 10^{-26}$) compared with those in the lowest quartile.

Interpretation Variants near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport affect vitamin D status. Genetic variation at these loci identifies individuals who have substantially raised risk of vitamin D insufficiency.

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Introduction

Vitamin D insufficiency affects as many as half of otherwise healthy adults in developed countries.¹ The musculoskeletal consequences of inadequate vitamin D concentrations are well established, and include childhood rickets, osteomalacia, and fractures.² A growing number of other disorders have also been linked to vitamin D insufficiency, although causal associations have not yet been established in randomised trials. These extraskeletal disorders include type 1 and type 2 diabetes,^{3–4} cardiovascular disease,^{5,6} increased risk of falls,⁷ and cancers of the breast, colon, and prostate.^{8–10} Results of a 2007 meta-analysis suggested that vitamin D supplementation substantially reduced mortality.¹¹

Personal, social, and cultural factors are important determinants of vitamin D availability via their effects on sun exposure and diet. Sufficient exposure to ultraviolet light or adequate intake from diet or supplements is needed to maintain vitamin D status. Concentrations of the widely accepted biomarker for vitamin D, 25-hydroxyvitamin D, are highest in the summer and lowest in the winter in northern latitudes. However, only about a quarter of the interindividual variability in 25-hydroxyvitamin D concentration is attributable to season of measurement, geographical latitude, or reported vitamin D intake.^{12,13} Results of previous twin and family studies suggest that genetic factors contribute substantially to this variability,^{13,14} with estimates of

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	Chromosome	Position	Nearest gene(s)	MAF	Combined p value for discovery samples (up to n=16 124)	Combined p value for replication samples (up to n=17 744)	Overall p value
rs2282679	4	72827247	GC	0.29	4.57×10 ⁻⁶³	2.88×10 ⁻⁴⁸	1.9×10 ⁻¹⁰⁹
rs3755967	4	72828262	GC	0.29	7.41×10 ⁻⁵³	3.00×10 ⁻²⁴	2.42×10 ⁻⁷⁵
rs17467825	4	72824381	GC	0.29	3.85×10 ⁻⁵²	1.61×10 ⁻²³	6.75×10 ⁻⁷⁴
rs1155563	4	72862352	GC	0.30	4.70×10 ⁻⁵⁵	4.26×10 ⁻²⁰	2.37×10 ⁻⁷³
rs2298850	4	72833131	GC	0.28	8.94×10 ⁻⁴⁹	2.12×10 ⁻²⁴	2.03×10 ⁻⁷¹
rs7041	4	72837198	GC	0.44	3.74×10 ⁻⁴²	1.78×10 ⁻¹⁸	6.31×10 ⁻⁵⁹
rs12785878	11	70845097	DHCR7/NADSYN1	0.23	1.27×10 ⁻¹²	2.39×10 ⁻¹⁶	2.12×10 ⁻²⁷
rs7944926	11	70843273	DHCR7/NADSYN1	0.23	1.56×10 ⁻¹³	7.57×10 ⁻⁴	8.96×10 ⁻¹⁶
rs12800438	11	70848651	DHCR7/NADSYN1	0.23	5.98×10 ⁻¹³	6.39×10 ⁻⁴	2.54×10 ⁻¹⁵
rs3794060	11	70865327	DHCR7/NADSYN1	0.23	8.09×10 ⁻¹³	6.44×10 ⁻⁴	3.38×10 ⁻¹⁵
rs4945008	11	70898896	DHCR7/NADSYN1	0.24	8.98×10 ⁻¹³	6.11×10 ⁻⁴	4.55×10 ⁻¹⁵
rs4944957	11	70845683	DHCR7/NADSYN1	0.23	1.43×10 ⁻¹²	7.36×10 ⁻⁴	8.70×10 ⁻¹⁵
rs10741657	11	14871454	CYP2R1	0.40	3.91×10 ⁻⁸	2.09×10 ⁻³⁴	3.27×10 ⁻²⁰
rs2060793	11	14871886	CYP2R1	0.40	2.69×10 ⁻⁶	2.36×10 ⁻⁷	1.73×10 ⁻¹¹
rs1993116	11	14866810	CYP2R1	0.40	2.94×10 ⁻⁶	1.28×10 ⁻⁶	6.25×10 ⁻¹¹
rs12794714	11	14870151	CYP2R1	0.43	6.24×10 ⁻⁵	8.71×10 ⁻⁷	1.84×10 ⁻⁹
rs10500804	11	14866849	CYP2R1	0.43	7.43×10 ⁻⁵	1.12×10 ⁻⁶	2.67×10 ⁻⁹
rs7116978	11	14838347	CYP2R1	0.36	1.17×10 ⁻⁵	7.59×10 ⁻⁵	4.99×10 ⁻⁹

Results within each locus are ordered by strength of association with 25-hydroxyvitamin D concentration. MAF=minor allele frequency.

Table 1: Single nucleotide polymorphisms identified in genome-wide association analyses for 25-hydroxyvitamin D concentrations

heritability as high as 53%. Although several rare mendelian disorders cause functional vitamin D insufficiency, data for the effect of common genetic variation on vitamin D status are scarce. Candidate gene studies have been done to examine the effect of specific vitamin D pathway genes, but these studies have been limited by small sample sizes and the small numbers of variants examined.¹⁵⁻¹⁸

The SUNLIGHT consortium (Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits) was formed in 2008. It represents a collaboration of cohorts from the UK, USA, Canada, Netherlands, Sweden, and Finland. We aimed to identify common genetic variants affecting vitamin D concentrations and risk of vitamin D insufficiency.

Methods
Participants

We undertook a large, multicentre, genome-wide association study of 15 cohorts in Europe, Canada, and the USA. The discovery sample consisted of 16 125 individuals of European descent drawn from five epidemiological cohorts: the Framingham Heart Study, TwinsUK, the Rotterdam Study, the 1958 British Birth Cohort (1958BC), and the Amish Family Osteoporosis Study (AFOS). Five additional cohorts (n=9367) with genome-wide association data were used for in-silico replication: the Cardiovascular Health Study, the North Finland Birth Cohort 1966 (NFBC1966), the Indiana cohort, the Health, Aging, and Body Composition study (Health ABC), and the Gothenburg Osteoporosis and

Obesity Determinants study (GOOD). We also undertook genotyping of selected variants in 5789 participants from four additional epidemiological cohorts (Canadian Multicentre Osteoporosis Study [CaMos], Chingford, Hertfordshire, and the Aberdeen Prospective Osteoporosis Screening Study [APOSS]), and 2715 additional participants from one of the discovery cohorts (1958BC). Full descriptions of all participating cohorts are shown in the webappendix (pp 1-7). Written informed consent was obtained from all participants in the included cohorts, and the study protocols were reviewed and approved by local institutional review boards.

Procedures

Details of genotyping methods, quality control, and imputation procedures used in all participating cohorts are shown in the webappendix (pp 7-14). 25-hydroxyvitamin D concentrations were measured by radioimmunoassay or chemiluminescent assay (DiaSorin Inc, Stillwater, MN, USA) in the Framingham Heart Study, TwinsUK, Rotterdam Study, Health ABC, AFOS, the GOOD cohort, and CaMoS. Detection limits ranged from 4 nmol/L to 10 nmol/L. In the 1958BC samples, 25-hydroxyvitamin D was measured with automated application of the ImmunoDiagnostic Systems OCTEIA ELISA on a Dade-Behring BEP2000 analyser (sensitivity of 5.0 nmol/L; Marburg, Germany).¹⁹ In the Cardiovascular Health Study, NFBC1966, the Indiana cohort, Chingford, Hertfordshire, and APOSS, total 25-hydroxyvitamin D was measured with high-performance liquid chromatography-tandem mass

spectrometry. Serum concentrations of vitamin D binding protein were measured with immunonephelometric assay in the TwinsUK cohort.²⁰ The detection limit was 50 mg/L.

Statistical analyses

At the threshold $\alpha=5\times 10^{-8}$, with a conservative discovery sample size of 14000, our study had 80% power to detect single nucleotide polymorphisms accounting for 0.28% of the total variance in 25-hydroxyvitamin D concentrations, and 90% power to detect polymorphisms accounting for 0.32% of the total variance.

Genome-wide analyses were done within every cohort. In the Framingham Heart Study, TwinsUK, the Rotterdam Study, 1958BC, AFOS, NFBC1966, the Indiana cohort, Health ABC, and the GOOD study, linear regression models were used to generate cohort-specific residuals of naturally log transformed 25-hydroxyvitamin D concentrations adjusted for age, sex, body-mass index (BMI), and season. Log transformation was used to reduce skewness in the distribution of 25-hydroxyvitamin D. We modelled season using categorical variables for summer (July–September), autumn (October–December), winter (January–March), and spring (April–June). One set of definitions was used for season because most cohorts were at similar latitudes, and all were in the northern hemisphere.

In cohorts that included related individuals (Framingham, TwinsUK, AFOS, Indiana Women), we assessed association between additively coded single nucleotide polymorphism genotypes and standardised 25-hydroxyvitamin D residuals using either linear mixed-effect models or the score test implemented in MERLIN (version 1.1.2).²¹ For imputed single nucleotide polymorphisms, expected number of minor alleles (ie, dose) was used in assessments of association between genotype and 25-hydroxyvitamin D residuals. In the Cardiovascular Health Study, analyses were adjusted for age, sex, and study site by inclusion of these factors as covariates in the model. In all samples, the genomic control approach was used to adjust p values for potential effects of mild population stratification and to prevent inflation of type I error occurring from any departure from normality of the trait variable.

A priori, we designated the first five genome-wide association studies, all of which used immunoassays to measure 25-hydroxyvitamin D concentrations, as discovery samples. The remaining five studies, three of which measured 25-hydroxyvitamin D by mass spectrometry and two by immunoassay, were designated as in-silico replication samples. We selected single nucleotide polymorphisms for replication if they had meta-analytic p values for association with 25-hydroxyvitamin D concentrations that were lower than 5×10^{-8} in the discovery samples. Additionally, we considered polymorphisms at or near six prespecified vitamin D pathway candidate genes: vitamin D receptor

	Framingham Heart Study (n=5656)	1958 British Birth Cohort (n=6552)
GC*		
Major homozygotes (nmol/L)	82.6 (0.73)	61.9 (0.34)
Heterozygotes (nmol/L)	74.8 (0.81)	57.0 (0.32)
Minor homozygotes (nmol/L)	64.6 (1.79)	52.8 (0.28)
DHCR7†		
Major homozygotes (nmol/L)	79.7 (0.71)	59.6 (0.32)
Heterozygotes (nmol/L)	76.3 (0.86)	56.3 (0.31)
Minor homozygotes (nmol/L)	71.7 (2.01)	55.7 (0.31)
CYP2R1‡		
Major homozygotes (nmol/L)	75.4 (0.87)	56.8 (0.31)
Heterozygotes (nmol/L)	78.6 (0.76)	60.2 (0.34)
Minor homozygotes (nmol/L)	81.6 (1.26)	61.1 (0.36)
Season		
Winter (nmol/L)	61.6 (1.00)	43.2 (0.26)
Spring/autumn (nmol/L)	77.4 (0.68)	57.1 (0.30)
Summer (nmol/L)	95.8 (1.00)	71.7 (0.31)
Supplementation		
Yes (nmol/L)	83.4 (0.80)	65.9 (0.32)
No (nmol/L)	74.7 (0.69)	56.9 (0.30)

Data are mean (SE). Sample from 1958 British Birth Cohort (1958BC) consists of a combination of the genome-wide association study sample and the de novo genotyping sample (webappendix p 2). *rs2282679 in Framingham cohort, rs4588 in 1958BC (r^2 between single nucleotide polymorphisms >0.99). †rs7944926 in Framingham cohort, rs12785878 in 1958BC (r^2 between polymorphisms >0.99). ‡rs10741657 in Framingham cohort and 1958BC.

Table 2: Mean 25-hydroxyvitamin D concentrations by genotype, season, and supplementation status

(VDR), 1- α -hydroxylase (*CYP27B1*), 25-hydroxylase (*CYP2R1*), 24-hydroxylase (*CYP24A1*), vitamin D binding protein (*GC*), and 27-hydroxylase and 25-hydroxylase (*CYP27A1*). These polymorphisms were tested in the replication samples if they met a p value threshold of 10^{-3} in the discovery samples. Lastly, we assessed selected polymorphisms for 25-hydroxyvitamin D association in the de-novo replication samples, using the same analytic approach. We then generated combined p values across the 15 studies.²²

We undertook the meta-analysis using a weighted Z-score-based approach, as implemented in the software METAL (version 2009-10-10). In this approach, association p values were converted to signed Z statistics, for which the sign showed the direction of effect with respect to a reference allele. All Z scores were assigned a weight proportional to the square root of the sample size. Weighted Z statistics were summed across studies to obtain a global Z score and a corresponding two-sided p value. We regarded p values lower than 5×10^{-8} as genome-wide significant.²³

We also assessed whether selected genetic variants from the continuous trait analyses were associated with vitamin D insufficiency in the Framingham Heart Study, TwinsUK, CaMoS, and 1958BC. We used two thresholds

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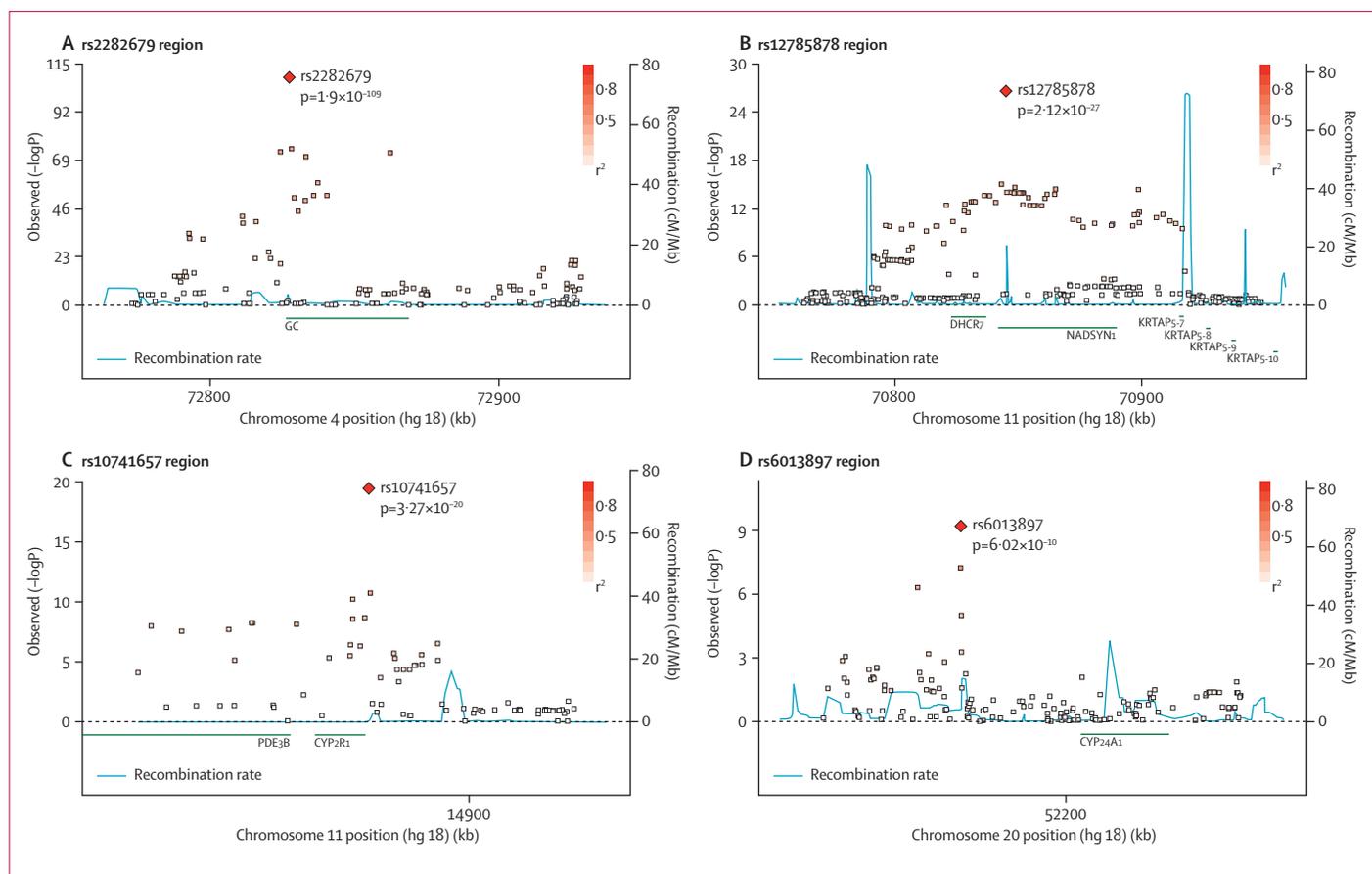


Figure 1: Regional linkage disequilibrium plots for single nucleotide polymorphisms at GC (A), *DHCR7/NADSYN1* (B), *CYP2R1* (C), and *CYP24A1* (D)

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for vitamin D insufficiency: 25-hydroxyvitamin D concentrations lower than 75 nmol/L (30 ng/mL) and lower than 50 nmol/L (20 ng/mL).¹ Covariates were age, sex, season, and BMI. We combined effect estimates from the logistic regression analysis across cohorts by meta-analysis using an inverse-variance weighting approach. We also did analyses using a 25 nmol/L (10 ng/mL) threshold, to examine whether genetic variants were associated with severe vitamin D deficiency.

Additionally, we constructed a genotype score by taking a weighted average of the number of risk alleles for members of a cohort, with weights established using β coefficients from the meta-analysis. Logistic regression was used to calculate the odds of vitamin D insufficiency according to quartile of the genotype score. For this analysis, we combined data from the Framingham Heart Study, TwinsUK, and 1958BC using a multivariate approach, with β coefficients for each quartile of genotype score meta-analysed jointly, as previously described.²⁴

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full

access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Characteristics of the study cohorts are summarised in the webappendix (pp 15–17). Table 1 shows the results of genome-wide association analyses. In analysis of data from the five discovery samples, single nucleotide polymorphisms at three unique loci met the prespecified threshold for genome-wide significance: 4p12, 11q12, and 11p15. The 4p12 polymorphisms were within or near the *GC* gene, and the results included a non-synonymous polymorphism in this gene, rs7041. The 11q12 polymorphisms were near *DHCR7/NADSYN1* (7-dehydrocholesterol reductase/NAD synthetase 1) and the 11p15 polymorphisms near *CYP2R1* (cytochrome P450, subfamily IIR).

Associations at all three loci were confirmed in replication samples. The polymorphism at *GC* with the lowest p value in discovery samples, rs2282679, had a combined p value of 2.9×10^{-48} in in-silico replication samples, with a consistent direction of effect. Additional genotyping was not done for this polymorphism. Polymorphism rs10741657 at *CYP2R1* had a p value of

2.1×10^{-14} in in-silico and de-novo replication samples, also with a consistent direction of effect. At the *DHCR7/NADSYN1* locus, a perfect proxy for rs7944926 (rs12785878, $r^2=1.0$) was genotyped in de-novo replication samples, and had a combined replication p value (in-silico and de-novo samples) of 2.4×10^{-16} . Overall p values (discovery and replication samples) for the three confirmed single nucleotide polymorphisms ranged from 3.3×10^{-20} to 1.9×10^{-109} (table 1). Figure 1 shows regional plots for the results at each locus. In the discovery cohorts, single nucleotide polymorphisms at the three confirmed loci (*GC*, *DHCR7/NADSYN1*, and *CYP2R1*) accounted for 1–4% of the variation in 25-hydroxyvitamin D concentrations.

We compared mean concentrations of 25-hydroxyvitamin D by genotype category at the three loci in the two largest cohorts (combined $n=12208$) with mean concentrations by supplementation status and season (table 2). Differences in mean 25-hydroxyvitamin D concentrations between minor and major homozygotes for the strongest genetic variants were similar to those seen with supplementation in these cohorts, and were nearly as large as differences recorded for a one season change.

In the candidate gene analysis, polymorphism rs6013897 near *CYP24A1* (cytochrome P450, family 24, subfamily A) had a p value of 7.2×10^{-4} in the discovery cohorts, and was tested for replication. The p value was 8.4×10^{-8} in the replication cohorts, resulting in an overall p value (discovery and replication) of 6.0×10^{-10} . Figure 1 shows a regional plot for the results at the *CYP24A1* locus. An additional candidate polymorphism, rs2544037 near *VDR*, had a p value of 6.2×10^{-4} in the discovery cohorts, but was not confirmed in replication samples. No polymorphisms were identified near *CYP27B1* or *CYP27A1* with p values less than 10^{-3} in the discovery cohorts.

We did additional analyses to assess effects of the three confirmed variants on risk of clinical vitamin D insufficiency (25-hydroxyvitamin D concentrations <75 nmol/L or <50 nmol/L). Table 3 shows results for the variants individually and in combination. Participants with a genotype score (combining the three variants) in the top quartile had increased odds of vitamin D insufficiency (figure 2). Genotype score was also associated with risk of severe vitamin D deficiency (25-hydroxyvitamin D concentration <20 nmol/L), with an adjusted odds ratio for participants in the top quartile of 1.43 (95% CI 1.13–1.79; $p=0.002$).

In view of the strong association of genetic variants at *GC* with 25-hydroxyvitamin D concentrations, we also examined whether these variants were associated with serum concentrations of vitamin D binding protein, which was measured in 1674 individuals in the TwinsUK cohort. The single nucleotide polymorphism rs2282679 was strongly associated with concentrations of vitamin D binding protein ($p=4.0 \times 10^{-42}$), with the minor allele related to reduced protein concentrations.

	25-hydroxyvitamin D concentration <75 nmol/L		25-hydroxyvitamin D concentration <50 nmol/L	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Individual variants				
<i>GC</i> (rs2282679)	1.63 (1.53–1.73)	3.5×10^{-50}	1.49 (1.40–1.59)	7.5×10^{-33}
<i>DHCR7</i> (rs7944926)	1.21 (1.14–1.29)	4.1×10^{-10}	1.21 (1.14–1.29)	4.7×10^{-99}
<i>CYP2R1</i> (rs10741657)	1.21 (1.14–1.29)	9.4×10^{-11}	1.06 (1.00–1.13)	0.06
Genotype score				
Quartile 1	1.0 (Reference)	..	1.0 (Reference)	..
Quartile 2	1.29 (1.15–1.46)	..	1.10 (0.97–1.25)	..
Quartile 3	1.56 (1.39–1.75)	..	1.38 (1.22–1.57)	..
Quartile 4	2.47 (2.20–2.78)*	..	1.92 (1.70–2.16)*	..

For individual variants, odds ratios are per copy of the risk allele. All logistic regressions were adjusted for age, sex, body-mass index, and season. *p values for trends in odds ratios for genotype scores were 2.3×10^{-48} for 25-hydroxyvitamin D concentrations lower than 75 nmol/L and 1.0×10^{-16} for lower than 50 nmol/L.

Table 3: Genetic variants and risk of vitamin D insufficiency

Discussion

Vitamin D insufficiency has been implicated in many musculoskeletal and extraskeletal diseases,^{1,2} which has led to substantial interest in the determinants of vitamin D status. Our findings establish a role for common genetic variants in regulation of circulating 25-hydroxyvitamin D concentrations. The presence of harmful alleles at the three confirmed loci more than doubled the risk of vitamin D insufficiency. These findings improve our understanding of vitamin D homeostasis and could assist identification of a subgroup of the white population who are at risk of vitamin D insufficiency.

DHCR7/NADSYN1 is a novel locus for association with vitamin D status, but one with compelling biological plausibility. *DHCR7* encodes the enzyme 7-dehydrocholesterol (7-DHC) reductase, which converts 7-DHC to cholesterol, thereby removing the substrate from the synthetic pathway of vitamin D₃, a precursor of 25-hydroxyvitamin D₃. Rare mutations in *DHCR7* lead to Smith-Lemli-Opitz syndrome, which is characterised by reduced activity of 7-DHC reductase, accumulation of 7-DHC, low cholesterol, and many congenital abnormalities.²⁵ Mutations in *DHCR7* might also confer a competitive advantage to heterozygous carriers, because high concentrations of 7-DHC could provide protection against rickets and osteomalacia from hypovitaminosis D.²⁶ However, few data exist for vitamin D status in individuals with Smith-Lemli-Opitz syndrome or carriers of mutations.²⁷ The finding that common variants at *DHCR7* are strongly associated with circulating 25-hydroxyvitamin D concentrations suggests that this enzyme could have a larger role in regulation of vitamin D status than has previously been recognised.

The gene at the second locus, *CYP2R1*, encodes a hepatic microsomal enzyme. *CYP2R1* could be the enzyme underlying 25-hydroxylation of vitamin D in the liver, but this suggestion is uncertain because many other

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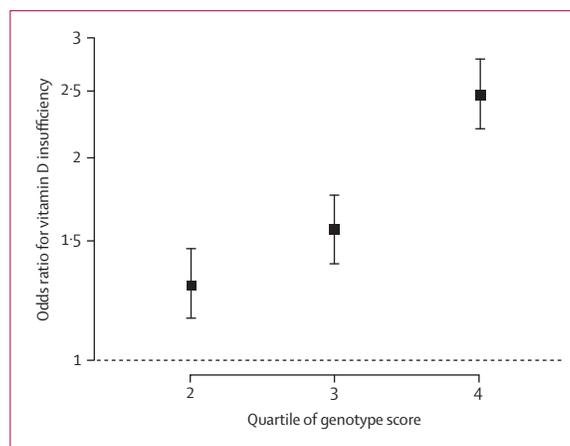


Figure 2: Risk of vitamin D insufficiency*, by quartile of genotype score
 *25-hydroxyvitamin D concentration lower than 75 nmol/L. Error bars show 95% CIs.

enzymes with 25-hydroxylase activity in vitro have been described.²⁸ Previous clinical studies have been limited to a case report of a Nigerian man with a point mutation in *CYP2R1* who had a history of rickets,²⁸ and a previous candidate gene study in 133 individuals with type 1 diabetes.¹⁸ Because affected individuals with *CYP2R1* polymorphisms have been difficult to identify, redundancy in the enzymes involved in the 25-hydroxylation step has been proposed. Thus, our finding that common variants at the *CYP2R1* locus are associated with circulating 25-hydroxyvitamin D concentrations is the strongest evidence so far that *CYP2R1* is the enzyme underlying the crucial first step in vitamin D metabolism.

The third gene, *GC*, encodes vitamin D binding protein, which is a 52–59 kDA protein synthesised in the liver that binds and transports vitamin D and its metabolites (including 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D).²⁹ A few investigators have reported associations between non-synonymous single nucleotide polymorphisms in this gene^{15–17,30,31} and 25-hydroxyvitamin D concentrations. However, their studies were small (≤ 1500 participants) and results were not replicated. The most widely studied *GC* variants are the non-synonymous polymorphisms rs7041 (Asp→Glu) and rs4588 (Thr→Lys). The previous nomenclature for *GC* haplotypes (GC1S, GC1F, and GC2) was based on specific combinations of alleles at these non-synonymous polymorphisms.¹⁵ Our data strongly confirm the association of rs7041 with circulating 25-hydroxyvitamin D. The other variant, rs4588, is not in the HapMap dataset and is thus not part of our imputed results. However, rs4588 is only 11 bp away from rs7041, and direct genotyping of rs4588 in one of our samples (TwinsUK) confirms that it is in linkage disequilibrium ($r^2 > 0.99$) with several associated variants from our genome-wide association study.

We also showed that *GC* variants associated with low 25-hydroxyvitamin D concentrations were strongly

related to reduced concentrations of vitamin D binding protein. Whether variation in the amount of circulating binding protein affects metabolism and availability of vitamin D is not well established. Concentrations of the binding protein have been postulated to affect delivery of 25-hydroxyvitamin D and activated vitamin D (1,25-dihydroxyvitamin D) to target organs, as well as clearance of vitamin D metabolites from the circulation.^{15,16} Alternatively, changes in quantity or function of the binding protein could be accompanied by changes in the relative proportions of free and bound 25-hydroxyvitamin D, with the free proportion being the potential rate-limiting factor for 1,25-dihydroxyvitamin D production. Further studies are needed to assess the effects of variation in serum concentrations of vitamin D binding protein.

In a screen of candidate gene variants, we noted an additional association at the locus containing *CYP24A1* that was genome-wide significant in pooled analyses of the discovery and replication samples. *CYP24A1* encodes 24-hydroxylase, which initiates degradation of both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. In previous candidate gene and linkage studies, investigators have not shown an association of variants at this locus with 25-hydroxyvitamin D concentrations, but these studies were relatively small.^{30,32}

A high genotype score for the three variants identified in our genome-wide association study conferred roughly a two-fold increase in risk of vitamin D insufficiency (25-hydroxyvitamin D concentrations < 50 nmol/L or < 75 nmol/L) compared with a score in the lowest quartile, after we accounted for environmental factors. This result suggests that variation at a few genetic loci could have a clinically important effect on risk of vitamin D insufficiency. High genotype score was associated with a 1.4-fold raised risk of severe vitamin D deficiency (< 25 nmol/L). Whether the reduced odds ratio for the 25 nmol/L threshold shows an increased contribution of environmental factors to the most severe forms of vitamin D deficiency is unclear, because severe deficiency was rare in our community-based cohorts.

Whether genetic predisposition modifies response to sun exposure or dietary supplementation warrants further study, especially in view of the large interindividual differences that have been reported in response to treatment with identical doses of vitamin D.³³ Furthermore, these variants might provide useful genetic approaches to investigate the role of vitamin D insufficiency in several chronic diseases with which this disorder has been epidemiologically linked.

The validity of our findings is lent support by the large study sample (more than 30 000 participants combined in discovery and validation samples), consistent results across several standard assays for 25-hydroxyvitamin D, and the strong biological plausibility of genes at the principal loci. Several limitations of the study also deserve mention, however. The study was not designed to identify

uncommon or rare variants. Resequencing at selected loci, partly on the basis of our results, could be used to identify uncommon variants with potentially large effects.

We used a multistage design to achieve maximum homogeneity of the assays used in the discovery analyses. We might have identified more genome-wide significant associations had we combined all study cohorts into one stage, but we would not have had a large replication sample. Other factors that might have contributed to reduced statistical power are second-order interactions (eg, with age) and the use of a stringent p-value threshold in the discovery stage.³⁴ Accordingly, the absence of specific candidate genes, such as those affecting vitamin D action or skin pigmentation, from our most significant results does not exclude an effect of genetic variation at these loci on vitamin D concentrations, but their contributions might be small compared with those of the genes that we identified.

Assays used to measure 25-hydroxyvitamin D concentrations varied between cohorts. To keep potential variability introduced by cohort-specific measurement techniques to a minimum, we standardised 25-hydroxyvitamin D concentrations within cohorts and analysed this variable as a continuous trait. Furthermore, primary results were meta-analysed with a Z-score-weighted approach, which is not scale-dependent. Specific information about dietary intake and sunlight exposure was not available from all cohorts. Such factors probably contribute to non-genetic variability in 25-hydroxyvitamin D concentrations, which would reduce the effect noted in our analyses.

The single nucleotide polymorphisms that we have identified might not be causal variants, but rather be in linkage disequilibrium with these variants. We did not examine downstream markers of vitamin D status, because 25-hydroxyvitamin D concentration is regarded as the most reliable indicator of vitamin D status. Other molecules, such as 1,25-dihydroxyvitamin D or parathyroid hormone, have greater intraindividual variability than does 25-hydroxyvitamin D and are affected by several determinants other than vitamin D status. Lastly, we studied only white individuals of European descent. Whether the genetic variants we identified affect vitamin D status in other racial or ethnic groups is unknown and warrants further study.

Contributors

JD, FZ, JBR, BK, JBM, DB, CO, DLK, JDC, PFO, NLG, LV, JS, MM, BK, KR, ML(1), ML(2), LJH, ALH, GZ, RJFL, and TF took part in data analysis. TJW, JBR, BK, JBM, DB, DPK, CO, MRJ, FR, DG, NJW, NKA, CC, ALH, ED, CP, NS, ML(2), TBH, AH, AGU, LP, DK, SBK, JCF, JAT, EH, TDS contributed to study design. TJW, FZ, DB, DPK, EAS, CO, DLK, MRJ, PFO, DKH, LV, MP, MIM, PA, MM, DJS, GLB, DG, NH, NJW, DH, NKA, CC, WDF, GZ, HMM, RJFL, DMR, AH, ED, YL, CP, HES, LJ, NS, JB, ML(2), TBH, JJ, JAC, LP, DSS, MJE, SBK, JAT, EH, TDS, SLH, QG, and SLB contributed to data collection. TJW, FZ, JBR, BK, JBM, DB, DPK, EAS, CO, DLK, MRJ, JDC, PFO, DKH, NLG, LV, MP, FR, MIM, IHB, DJS, SLB, PFJ, GLB, CLC, MW, KR, DG, NH, ML(1), NJW, LJH, NKA, CC, SM, ALH, HMM, RJFL, AH, ED, CP, HES, BMP, ML(2), TBH,

AH, JJ, JAC, AGU, LP, DK, MJE, SBK, JCF, JAT, JD, EH, and TDS interpreted results. FZ, JBR, JBM, DB, DPK, EAS, CO, JDC, PFO, DKH, NLG, LV, MP, FR, MIM, PA, IHB, DJS, SLB, PFJ, GLB, MG, CLC, MW, KR, DG, NH, ML(1), NJW, LJH, NKA, CC, SM, WDF, ALH, HMM, RJFL, ED, YL, CP, HES, BMP, ML(2), TF, TBH, AH, JJ, JAC, AGU, DK, DSS, MJE, SBK, JCF, JAT, JD, EH, and TDS read the manuscript critically. The writing group consisted of TJW, FZ, JBR, BK, EAS, DLK, MRJ, MG, JCF, JAT, JD, EH, and TDS. ML(1)=Martin Ladouceur. ML(2)=Mattias Lorentzon.

Conflicts of interest

TJW has served on the scientific advisory board of Diasorin. DKH has received honoraria from Abbott Nutrition. MW has received consultancy fees, honoraria, and speakers' fees from Abbott and Genzyme. DMR has acted as a consultant for Novartis, Roche, Pfizer, Amgen, Shire, Merck, and Servier, has received speakers' fees from Novartis, Roche, and Amgen, and owns stock in GlaxoSmithKline and Astra Zeneca. ML(2) has received lecture fees from Novartis and Sanofi-Aventis. All other authors declare that they have no conflicts of interest.

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For the Framingham Heart Study see <http://www.framinghamheartstudy.org>

For the Wellcome Trust Case-Control Consortium website see www.wtccc.org.uk

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For the Cardiovascular Health Study website see <http://www.chs-nhlbi.org/pi.htm>

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