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

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Abstract

Background—Vitamin D is crucial for maintaining musculoskeletal health. Recently, vitamin D insufficiency has been linked to a number of extraskeletal disorders, including diabetes, cancer, and cardiovascular disease. Determinants of circulating 25-hydroxyvitamin D (25-OH D) include sun exposure and dietary intake, but its high heritability suggests that genetic determinants may also play a role.

Methods—We performed a genome-wide association study of 25-OH D among ~30,000 individuals of European descent from 15 cohorts. Five cohorts were designated as discovery cohorts (n=16,125), five as *in silico* replication cohorts (n=9,366), and five as *de novo* replication

cohorts (n=8,378). Association results were combined using z-score-weighted meta-analysis. Vitamin D insufficiency was defined as 25-OH D <75 nmol/L or <50 nmol/L.

Findings—Variants at three loci reached genome-wide significance in the discovery cohorts, and were confirmed in the 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*); 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). A genotype score was constructed using the three confirmed variants. Those in the top quartile of genotype scores had 2- to 2.5-fold elevated odds of vitamin D insufficiency ($P \leq 1 \times 10^{-26}$).

Interpretation—Variants near genes involved in cholesterol synthesis (*DHCR7*), hydroxylation (*CYP2R1, CYP24A1*), and vitamin D transport (*GC*) influence vitamin D status. Genetic variation at these loci identifies individuals of European descent who have substantially elevated risk of vitamin D insufficiency.

Background

Vitamin D insufficiency affects as many as one-half of otherwise healthy adults in developed countries.[1] The musculoskeletal consequences of inadequate vitamin D are well-established, and include childhood rickets, osteomalacia, and fractures.[2] A growing number of other conditions have also been linked to vitamin D insufficiency, although causal associations have not yet been established in randomized trials. These extra-skeletal conditions include type 1 and 2 diabetes,[2-4] cardiovascular disease,[5,6] falls,[7] and cancers of the breast, colon, and prostate.[8-10] A recent meta-analysis suggested that vitamin D supplementation led to significant reductions in mortality.[11]

Personal, social, and cultural factors are important determinants of vitamin D status via their influence on sun exposure and diet. Maintaining vitamin D status requires sufficient exposure to ultraviolet light or adequate intake from diet or supplements. Levels of 25-OH D, the widely-accepted biomarker of vitamin D status, are highest in the summer and lowest in the winter in northern latitudes. However, only about a quarter of the inter-individual variability in 25-OH D is attributable to season of measurement, geographic latitude, or reported vitamin D intake.[12,13] Previous twin and family studies suggest that genetic factors contribute substantially to variability in 25-OH D,[13,14] with estimates of heritability as high as 53%. Although several rare Mendelian disorders cause functional vitamin D insufficiency, there are few data on the influence of common genetic variation on vitamin D status. Candidate gene studies have been performed to examine the effect of specific vitamin D-pathway genes, but these studies have been limited by modest samples sizes and the small numbers of variants examined.[15-18]

Thus, we conducted a large, multicentre genome-wide association study (GWAS) involving approximately 30,000 subjects from 15 cohorts in Europe, Canada, and the United States. Our aim was to identify common genetic variants influencing vitamin D levels and the risk of vitamin D insufficiency.

Methods

Study samples and genotyping

The discovery sample consisted of 16,125 individuals of European descent drawn from five epidemiological cohorts: the Framingham Heart Study, Twins UK, the Rotterdam Study, the 1958 British Birth Cohort (1958BC), and the Amish Family Osteoporosis Study (AFOS). There were five additional cohorts (n=9,366) with genome-wide association data used for *in silico* replication: the Cardiovascular Health Study, the North Finland Birth Cohort 1966

(NFBC1966), the Indiana cohort, the Dynamics of Health, Aging, Body, and Composition study (Health ABC), and the Gothenburg Osteoporosis and Obesity Determinants study (GOOD). We also performed genotyping of selected variants in 5,715 participants from four additional epidemiologic cohorts (Canadian Multicentre Osteoporosis Study [CaMos], Chingford, Hertfordshire, and the Aberdeen Prospective Osteoporosis Screening Study [APOSS]), and 2,715 additional participants from one of the discovery cohorts (1958BC). Full descriptions of all participating cohorts, as well as details of genotyping methods, quality control, and imputation procedures, are provided in the Supplemental Methods. Characteristics of the study samples are summarized in Supplemental Table 1.

25-OH D measurements

Concentrations of 25-OH D were measured by radioimmunoassay or chemiluminescent assay (DiaSorin Inc, Stillwater, MN) in the following cohorts: Framingham Heart Study, Twins UK, Rotterdam Study, Health ABC, AFOS, the GOOD cohort, and CaMoS. Detection limits ranged from 4 to 10 nmol/L. In the 1958BC samples, 25-OH D was measured using an automated application of the IDS OCTEIA ELISA on the Dade-Behring BEP2000 analyser (sensitivity of 5.0 nmol/L).[19] In the Cardiovascular Health Study, NFBC1966, the Indiana cohort, Chingford, Hertsfordshire, and APOSS, total 25-OH D was measured using high performance liquid chromatography-tandem mass spectrometry. Serum vitamin D binding protein (DBP) was measured by an immunonephelometric assay in the Twins UK cohort.[20] The detection limit was 50 mg/L.

Statistical analyses

Genome-wide analyses were conducted within each cohort. In the Framingham Heart Study, Twins UK, 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-OH D levels adjusted for age, sex, body mass index (BMI), and season. Log transformation was used to reduce skewness in the distribution of 25-OH D. Season was modeled using categorical variables for summer (July-September), fall (October-December), winter (January to March), and spring (April to June). A single set of definitions was used for season because the majority of the cohorts were at similar latitudes, and all of them were in the northern hemisphere. In cohorts that included related individuals (Framingham, Twins UK, AFOS, Indiana women), association between the additively-coded SNP genotypes and the standardized 25-OH D residuals was assessed using either linear mixed effect models or the score test implemented in MERLIN.[21] For imputed SNPs, expected number of minor alleles (i.e. dosage) was used in assessments of association between genotype and 25-OH D residuals. In the Cardiovascular Health Study, analyses were adjusted for age, sex, and study site by including each as a covariate in the model. In all samples, the genomic control approach was used to adjust the 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 GWAS, all of which used immunoassays for measuring 25-OH D levels, as the "discovery samples." The remaining five GWAS, three of which measured 25-OH D by mass spectrometry and two by immunoassay, were designated as *in silico* replication samples. We selected SNPs for replication if they had meta-analytic *P*-values $< 5 \times 10^{-8}$ in the discovery samples. Additionally, we considered SNPs at or near six pre-specified vitamin D pathway candidate genes: vitamin D receptor (*VDR*), 1-α-hydroxylase (*CYP27B1*), 25-hydroxylase (*CYP2R1*), 24-hydroxylase (*CYP24A1*), vitamin D binding protein (*GC, DBP*), and 27- and 25-hydroxlyase (*CYP27A1*). These SNPs were tested in the replication samples if they met a *P*-value threshold of 10^{-3} in the discovery samples. Lastly, selected SNPs were assessed for 25-OH D association in the *de novo*

replication samples, using the same analytic approach. We then generated combined *P*-values across the 15 studies.[22]

Meta-analysis was conducted using a weighted z-score based approach, as implemented in the software METAL (www.sph.umich.edu/csg/abecasis/metal/). In this approach, association *P*-values are converted to signed z-statistics, where the sign reflects the direction of effect with respect to a reference allele. Each z-score is assigned a weight proportional to the square root of the sample size. Weighted z-statistics are summed across studies to obtain a global z-score and a corresponding two-sided *P*-value. A *P*-value $< 5 \times 10^{-8}$ was considered 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, Twins UK, CaMoS, and 1958BC. Vitamin D insufficiency was defined using 2 cutpoints, 25-OH D < 75 nmol/L (30 ng/ml) and < 50 nmol/L (20 ng/ml).[1] Covariates included age, sex, season, and BMI. Effect estimates from the logistic regression analysis were combined across cohorts by meta-analysis using inverse-variance weighting approach. We also performed analyses using a lower cutpoint, 25 nmol/L (10 ng/ml), to examine whether genetic variants were associated with severe vitamin D deficiency.

Additionally, a genotype score was constructed by taking a weighted average of the number of risk alleles for members of a cohort, with the weights determined by the beta coefficients from the meta-analysis. Logistic regression was performed to determine the odds of vitamin D insufficiency according to quartile of the genotype score. For this analysis, data from the Framingham Heart Study, Twins UK, and 1958BC were combined using a multivariate approach, with beta coefficients for each quartile of genotype score meta-analysed jointly, as previously described.[24]

Results

Results of genome-wide association analyses are summarized in Table 1. In the analysis of data from the five discovery samples, SNPs at three unique loci met the pre-specified threshold for genome-wide significance: 4p12 (lowest $P=4.6 \times 10^{-63}$ for rs2282679), 11q12 ($P=1.6 \times 10^{-13}$ for rs7944926), and 11p15 ($P=3.9 \times 10^{-8}$ for rs10741657). The 4p12 SNPs were within or near the *GC* gene, and the top results included a non-synonymous SNP in this gene, rs7041 ($P=3.7 \times 10^{-42}$ for association with 25-OH D). The 11q12 and 11p15 SNPs were near *DHCR7/NADSYN1* (7-dehydrocholesterol reductase, NAD synthetase 1) and *CYP2R1* (cytochrome P450, subfamily IIR), respectively.

The associations at all three loci were confirmed in the replication samples. The SNP at *GC*, rs2282679, had a combined *P*-value of 2.9×10^{-48} in the *in silico* replication samples, with a consistent direction of effect. Additional genotyping for this SNP was not performed. SNP rs10741657 at *CYP2R1* had $P=2.1 \times 10^{-14}$ in the *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 the *de novo* replication samples. The combined replication *P*-value (*in silico* and *de novo* replication samples) for rs12785878 was 2.4×10^{-16}. Overall *P*-values (discovery and replication samples) for the three confirmed SNPs ranged from 3.3×10^{-20} to 1.9×10^{-109}, as shown in Table 1. Regional plots for the results at each locus are shown in Figures 1A-C. In the discovery cohorts, SNPs at the three confirmed loci (*GC*, *DHCR7/NADSYN1*, and *CYP2R1*) explained up to 1-4% of the variation in 25-OH D concentrations.

Table 2 compares the influence of the genetic variants at the 3 validated loci with the observed effects of supplementation and season. Means levels of 25-OH D by genotype

category at the 3 validated loci are shown from the 2 largest cohorts (N=12,208), along with mean levels by supplementation status and season. The relative differences in mean 25-OH D between minor and major homozygotes for the strongest genetic variants were comparable to those seen with supplementation in these cohorts, and nearly as large as those seen with a one season change.

In the candidate gene analysis, SNP 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}. A regional plot for the results at the *CYP24A1* locus is shown in Figure 1D. An additional candidate SNP, rs2544037 near *VDR*, had a *P*-value of 6.2×10^{-4} in the discovery cohorts, but failed to replicate in the replication samples. There were no SNPs near *CYP27B1* or *CYP27A1* with *P*-values less than 10^{-3} in the discovery cohorts.

We performed additional analyses to assess the influence of the three variants identified by GWAS on the risk of clinical vitamin D insufficiency (25-OH D < 75 nmol/L or < 50 nmol/L). Results for the variants, individually and in combination, are shown in Table 3. Individuals with a "genotype score" (combining the 3 variants) in the top quartile had a 2- to 2.5-fold increased odds of vitamin D insufficiency (Figure 2). The genotype score was also associated with the risk of severe vitamin D deficiency (25-OH D < 20 nmol/L), with an adjusted odds ratio in the top quartile of 1.43 (95% confidence interval, 1.13-1.79; p=0.002).

Given the strong association of genetic variants at *GC* with 25-OH D concentrations, we also examined whether these variants were associated with serum DBP, which was measured in 1,674 individuals in the Twins UK cohort. SNP rs2282679 was strongly associated with DBP ($P=4.0 \times 10^{-42}$), with the minor allele related to lower DBP concentrations.

Discussion

Vitamin D insufficiency has been implicated in a wide range of 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 the regulation of circulating 25-OH D levels. Indeed, the presence of deleterious alleles at the three confirmed loci more than doubled the odds of vitamin D insufficiency. These findings improve our understanding of vitamin D homeostasis and may assist in the identification of a subgroup of Caucasians at risk for 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 reductase, which converts 7-dehydrocholesterol (7-DHC) to cholesterol, thereby removing the substrate from the synthetic pathway of vitamin D3, a precursor of 25-OH D_3. Rare mutations in *DHCR7* lead to Smith-Lemli-Opitz syndrome, which is characterized by reduced activity of 7-dehydrocholesterol reductase, accumulation of 7-DHC, low cholesterol, and multiple congenital abnormalities.[25] It has been speculated that mutations in *DHCR7* also confer a competitive advantage to heterozygous carriers, because high levels of 7-DHC could provide protection against rickets and osteomalacia from hypovitaminosis D.[26] However, there are few data on vitamin D status in individuals with Smith-Lemli-Opitz syndrome or carriers of known mutations.[27] The finding that common variants at *DHCR7* are strongly associated with circulating 25-OH D suggests that this enzyme could play a larger role in the normal regulation of vitamin D status than previously recognized.

The gene at the second locus, *CYP2R1*, encodes a hepatic, microsomal enzyme first described in 2003. It has been suggested that CYP2R1 is the enzyme responsible for 25-hydroxylation of vitamin D in the liver, but this is uncertain because many other enzymes with 25-hydroxylase activity *in vitro* have been described.[28] Prior 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, it has been proposed that there is redundancy in the enzymes responsible for the 25-hydroxylation step. Thus, our finding that common variants at the *CYP2R1* locus are associated with circulating 25-OH D represents the strongest evidence to date that CYP2R1 is the enzyme responsible for the critical first step in vitamin D metabolism.

The third gene, *GC*, encodes DBP, a 52-59 kDA protein synthesized in the liver that binds and transports vitamin D and its metabolites (including 25-OH D and $1,25(OH)_2D$).[29] A few recent studies have reported associations between nonsynonymous SNPs in this gene,[15-17,30,31] and 25-OH D concentrations. However, these studies were relatively small (≤ 1500 subjects) and lacked replication. The most commonly studied *GC* variants are the nonsynonymous SNPs rs7041 (Asp→Glu) and rs4588 (Thr→Lys). The older nomenclature for *GC* haplotypes (GC1S, GC1F, and GC2) is based on specific combinations of alleles at these nonsynonymous SNPs.[15] Our data strongly confirm the association of rs7041 with circulating 25-OH D (overall meta-analytic $P=1.9 \times 10^{-109}$). The other variant, rs4588, is not in the HapMap dataset and 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 (Twins UK) confirms that it is in linkage disequilibrium ($r^2>0.99$) with multiple associated variants from our genome-wide association study.

We also showed that *GC* variants associated with lower 25-OH D concentrations were strongly related to lower levels of DBP. Whether variation in the amount of circulating DBP influences further metabolism and availability of vitamin D is not well established. It has been hypothesized that levels of DBP may affect the delivery of 25-OH D and activated vitamin D ($1,25(OH)_2D$) to target organs, as well as clearance of vitamin D metabolites from the circulation.[15,16] On the other hand, alterations in quantity or function of DBP could be accompanied by changes in the relative proportions of free and bound 25-OH D, with the free fraction being the potential rate-limiting factor for $1,25(OH)_2D$ production. Further studies are needed to assess the effects of variation in serum DBP.

In a screen of candidate gene variants, we observed an additional association at the locus containing *CYP24A1* that was genome-wide significant in pooled analyses of the discovery and replication samples ($P=6 \times 10^{-10}$). *CYP24A1* encodes 24-hydroxylase, which initiates the degration of both 25-OH D and $1,25(OH)_2D$. Prior candidate gene and linkage studies have failed to show an association of variants at this locus with 25-OH D levels, but these studies were small and based on highly-selected cohorts.[30,32]

A high "genotype score" using the three confirmed GWAS variants conferred a 2- to 2.5-fold risk of having vitamin D insufficiency to individuals in the upper quartile of the score compared with those in the lower quartile, after accounting for the usual environmental factors. This observation indicates that variation at a relatively small number of genetic loci could have a clinically-important impact on the risk of vitamin D insufficiency. The genotype score was also associated with an elevated risk of severe vitamin D deficiency (25-OH D < 25 nmol/L), although the odds ratio was slightly lower (1.4 in the highest quartile). It is unclear whether the lower odds ratio reflects a greater contribution of environmental factors to the most severe forms of vitamin D deficiency, because the prevalence of severe deficiency was low in our community-based cohorts.

Whether this genetic predisposition modifies the response to sun exposure or dietary supplementation warrants further study, particularly given the large inter-individual differences that have been observed in response to treatment with identical doses of vitamin D.[33] Furthermore, these variants might serve as useful genetic tools for studies in disease cohorts to determine whether vitamin D insufficiency plays a causal role in a variety of chronic diseases with which it has been epidemiologically linked.

The validity of our findings is supported by the large study sample (more than 30,000 subjects combined in discovery and validation samples), consistent results across several standard assays for 25-OH 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, based in part upon our results, could be used to identify less common variants of potentially larger effects.

We used a multi-stage design in order to maximize the homogeneity of the assays used in the discovery analyses. It is possible that more genome-wide significant associations would have been identified by combining all GWAS cohorts into a single stage, although we would have lacked a large replication sample. Other factors that may have contributed to reduced statistical power include second-order interactions (such as with age) and the use of a stringent p-value threshold in the discovery stage.[34] Accordingly, the absence of certain candidate genes from our top hits, such as those affecting vitamin D action or skin pigmentation, doesn't exclude a role for genetic variation at these loci in influencing vitamin D levels, but their contribution may be small compared with the genes identified.

Some cohorts used different assays for measurement of 25-OH D levels. To minimize the potential variability introduced by cohort-specific measurement techniques, we performed analyses in which 25-OH D levels were standardized within cohort and analyzed as a continuous trait. Furthermore, primary results were meta-analysed using a z-score weighted approach, which is not scale-dependent. Specific information on dietary intake and sunlight exposure was not available from all of the cohorts. Such factors likely contribute to non-genetic variability in 25-OH D concentrations, which would tend to reduce the effect observed in our analyses.

The identified SNPs may not be the causal variants, but rather in linkage disequilibrium with them. We did not examine “downstream” markers of vitamin D status, because 25-OH D concentrations are considered the most reliable indicators of vitamin D status. Other molecules, such as $1,25(OH)_2D$ or parathyroid hormone, have greater intra-individual variability and reflect the influence of multiple determinants in addition to vitamin D status. Lastly, we studied only white individuals of European descent. Whether the genetic variants identified in the present study influence vitamin D status in other racial/ethnic groups is unknown and warrants further study.

In conclusion, we report the results of the first large-scale, systematic investigation of genetic determinants of vitamin D insufficiency. Elucidating the genetic architecture of this trait provides a better understanding of the regulation of vitamin D metabolism. Additionally, the genetic variants described in this report identify individuals at substantially elevated risk for vitamin D insufficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1A

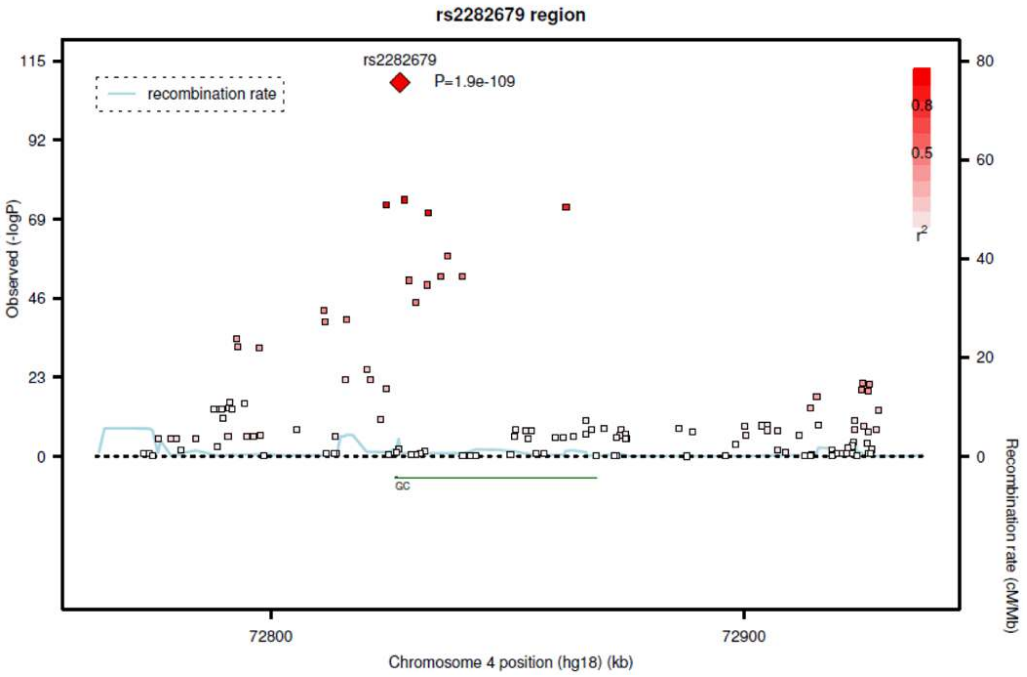

Figure 1B

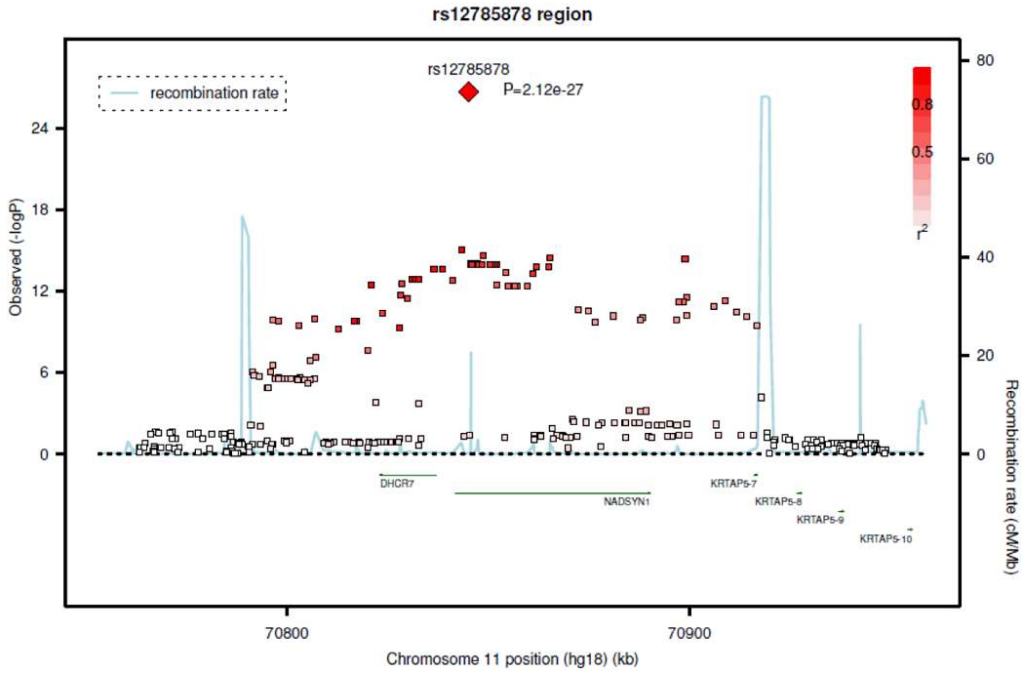

Figure 1C

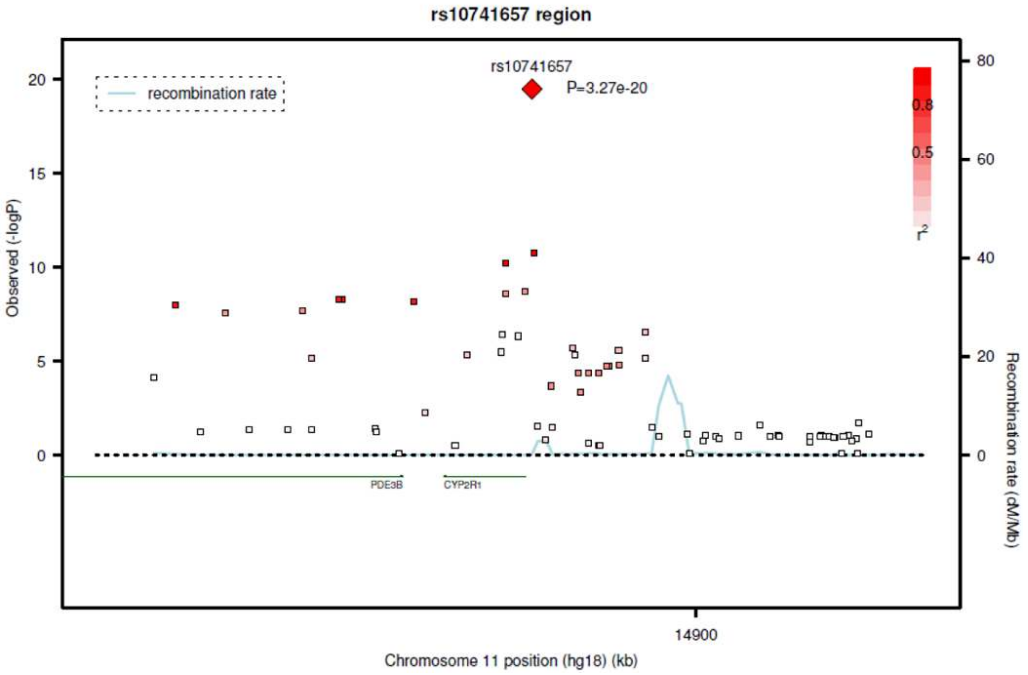

Figure 1D

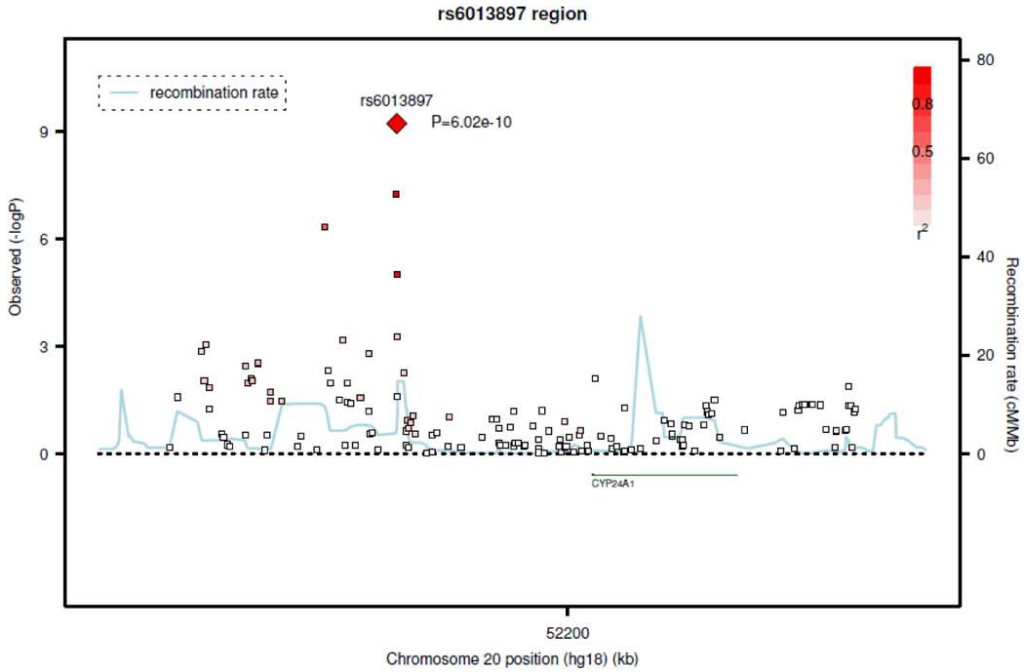

Figure 1.

Regional linkage disequilibrium plots for SNPs at *GC* (panel 1A), *DHCR7/NADSYN1* (panel 1B), *CYP2R1* (panel 1C), and *CYP24A1* (panel 1D).

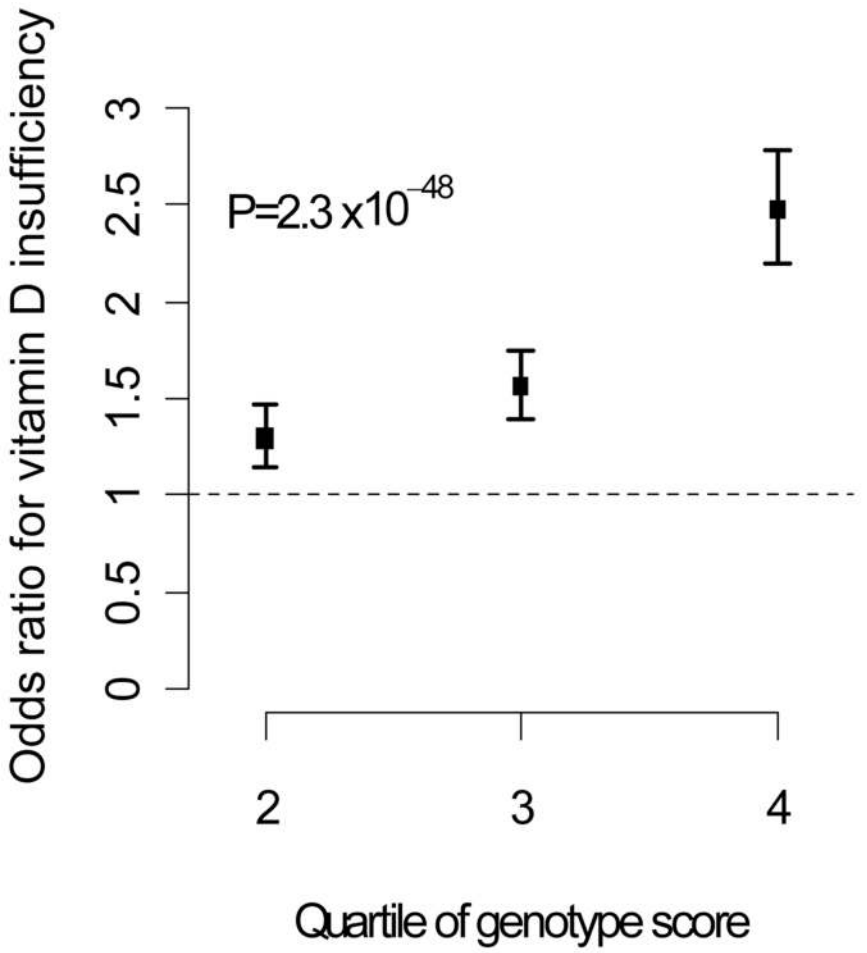

Figure 2.
Risk of vitamin D insufficiency (using threshold of 75 nmol/L), according to quartile of genotype score. Bars indicate 95% confidence intervals.

Table 1

Results of genome-wide association analyses for 25-OH D concentrations

SNP	Chrom	Position	Nearest gene(s)	MAF	Combined *P* Discovery samples (up to N=16,124)	Combined *P* Replication samples (up to N=17,744)	Overall *P*
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^{-14}	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}

The top 6 SNP results are shown at each chromosomal locus, ordered from smallest to largest overall *P*.

MAF: minor allele frequency.

Table 2
Mean 25-OH D levels by genotype, season, and supplementation status

	Framingham Heart Study (n=5,656)	1958 British Birth Cohort (n=6,552)
GC[*]		
Major homozygotes	82.6 (0.73)	61.9 (0.33)
Heterozygotes	74.8 (0.81)	57.0 (0.30)
Minor homozygotes	64.6 (1.79)	52.8 (0.27)
DHCR7[†]		
Major homozygotes	79.7 (0.71)	59.6 (0.31)
Heterozygotes	76.3 (0.86)	56.3 (0.30)
Minor homozygotes	71.7 (2.01)	55.7 (0.32)
CYP2R1[**]		
Major homozygotes	75.4 (0.87)	56.8 (0.34)
Heterozygotes	78.6 (0.76)	60.2 (0.32)
Minor homozygotes	81.6 (1.26)	61.1 (0.29)
Season		
Winter	61.6 (1.00)	43.2 (0.26)
Spring/Fall	77.4 (0.68)	57.1 (0.30)
Summer	95.8 (1.00)	71.7 (0.30)
Supplementation		
Yes	83.4 (0.80)	65.9 (0.30)
No	74.7 (0.69)	56.9 (0.31)

Means (SE) are shown, in nmol/L. Sample from 1958 British Birth cohort is combined from the GWAS sample and the *de novo* genotyping sample (see Supplementary Methods).

[*] rs2282679 in Framingham, rs4588 in 1958 Birth Cohort (r^2 between SNPs >0.99).

[†] rs7944926 in Framingham, rs12785878 in 1958 Birth Cohort (r^2 between SNPs >0.99).

[**] rs10741657 in Framingham and 1958 Birth Cohort.

Table 3

Genetic variants and risk of vitamin D insufficiency

	< 75 nmol/L		< 50 nmol/L	
	Odds ratio*	***P*-value**	**Odds ratio***	***P*-value**
Individual variants				
GC (rs2282679)	1.63 (1.53-1.73)	3.5×10^{-50}	1.49 (1.40-1.59)	7.5×10^{-33}
DHCR7 (rs7944926)	1.21 (1.14-1.29)	4.1×10^{-10}	1.21 (1.14-1.29)	4.7×10^{-09}
CYP2R1 (rs10741657)	1.21 (1.45-1.29)	9.4×10^{-11}	1.06 (1.00-1.13)	0.06
Genotype score				
Quartile 1	1.0 (Referent)		1.0 (Referent)	
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)	
***P*-for-trend**	2.3×10^{-48}		1.0×10^{-26}	

For individual variants, odds ratios are per copy of the "risk allele" with 95% confidence intervals shown in parentheses. All logistic regressions were adjusted for age, sex, body mass index, and season.