Genetic Risk Score for Serum 25-hydroxyvitamin D Concentration Helps to Guide Personalized Vitamin D Supplementation in Healthy Finnish Adults

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Abbreviations:

CYP24A1, cytochrome P450 family 24 subfamily A member 1

CYP2R1, cytochrome P450 family 2 subfamily R polypeptide 1

DHCR7, 7-dehydrocholesterol reductase

DHR, Digital Health Revolution

FFQ, food frequency questionnaire

FIMM, Institute for Molecular Medicine Finland

GC, GC vitamin D binding protein

GRS, genetic risk score

GWAS, genome-wide association study

H2011, Health 2011

HWE, Hardy-Weinberg equilibrium

MAF, minor allele frequency

M/m, major/minor allele

S25(OH)D, serum 25-hydroxyvitamin D

SNP, single nucleotide polymorphism

THL, Finnish Institute for Health and Welfare

25(OH)D, 25-hydroxyvitamin D

VDSP, Vitamin D Standardization Program

ABSTRACT[RS1]

Background: Genetic factors modify serum 25-hydroxyvitamin D (25(OH)D) concentration and affect the optimal individual intake levels of vitamin D.

Objective: We aimed to personalize vitamin D supplementation recommendations by applying knowledge of genetic factors affecting serum 25(OH)D.

Methods: We performed a genome-wide association study of serum 25(OH)D concentration in the Finnish Health 2011 cohort (*n* = 3,339) using multiple linear regression and developed a genetic risk score (GRS) for serum 25(OH)D. The population-matched GRS was applied to tailor vitamin D supplementation for 96 participants of a longitudinal Digital Health Revolution (DHR) Study. Serum 25(OH)D and dietary habits were monitored 3.5 months after personalized supplementation guidance. Serum 25(OH)D concentrations were measured using immunoassays, and the intake of vitamin D was assessed using food frequency questionnaires. Data analyses were performed using appropriate cross-sectional and repeated measures statistical tests and models.

Results: The *GC* and *CYP2R1* loci showed genome-wide significant associations with serum 25(OH)D and were used to develop a two-SNP (rs4588 and rs10741657) GRS. In the DHR Study, the fraction of daily vitamin D supplement users increased from 32.6% to 60.2% (*P* = 6.5×10^{-6}) and the mean serum 25(OH)D increased from 64.4 (± 20.9) nmol/L to 68.5 (± 19.2) nmol/L (*P* = 0.006) during the 3.5-month follow-up. Furthermore, the difference in the mean serum 25(OH)D between participants with no risk alleles and participants with three or

four risk alleles decreased from 20.7 nmol/L to 8.0 nmol/L (P = 0.0063). In the combined group of participants with three or four risk alleles, the percentage of individuals with serum 25(OH)D <50 nmol/L tended to decrease from 38.1% to 10.0% (P = 0.07).[RS2][JK3]

Conclusions: The GRS helped to identify individuals genetically predisposed to low serum 25(OH)D in the Finnish population. We propose that the direct return of data on individual genetic risk for low vitamin D status, serum 25(OH)D, and lifestyle factors could be used to guide personalized vitamin D supplementation and promote individualized health and that this model could be tailored to other populations and countries.

Keywords: Vitamin D; genetic risk score; personalized nutrition; DHR Study; Health 2011 Survey

INTRODUCTION

Vitamin D deficiency and insufficiency are major public health problems affecting more than a billion people worldwide (1-3). Vitamin D deficiency may cause rickets and osteomalacia and increase the risk of osteoporosis and bone fractures (4, 5). Low serum 25-hydroxyvitamin D (25(OH)D), which is the best available biomarker of vitamin D status, has also been associated with many other adverse health outcomes, such as cardiovascular disease, several cancers, diabetes, autoimmune diseases, and infections, as well as total mortality (4, 6-8). Although Mendelian randomization studies provide evidence for causal relationships (9-15), randomized controlled trials have so far largely failed to prove clinically relevant beneficial effects of vitamin D supplementation on non-skeletal health outcomes (7, 16-18).

Vitamin D deficiency is correctable but requires public health actions (3) and significant long-term interest and attention to personal health. Systematic vitamin D food fortification has been suggested as a possible solution to the problem (19), and it has already been successfully implemented in Finland (20, 21). Food fortification is probably a valid strategy to avoid very low serum 25(OH)D concentrations, i.e., below 25-30 nmol/L (22). If we want to have serum 25(OH)D concentration 50 nmol/L or higher in the majority of the population, systematic large-scale vitamin D supplementation would be needed as a public health action (22). However, there is substantial inter-individual heterogeneity in serum 25(OH)D concentrations in response to vitamin D supplementation (23, 24). Moreover, there is no scientific consensus on the optimal serum 25(OH)D concentration nor the definition of vitamin D deficiency (23, 25-28), and expert panels have not been able to agree on the optimal dietary intake levels of vitamin D (23, 25-29).

In the Nordic countries, the recommended intake of vitamin D is 10 μg/d from two weeks of life up to the age of 74 (29). This intake should maintain serum 25(OH)D concentration around 50 nmol/L for the majority of the population, taking into account the dark wintertime at Northern latitudes (29). Based on the national Health 2011 (H2011) Survey in Finland, the mean serum 25(OH)D concentration in the adult population was 65 nmol/L (20). However, 9% of those who did not use vitamin D supplements had serum 25(OH)D concentration below 50 nmol/L despite the consumption of fish, fortified fluid milk products, and fortified fat spreads as recommended by the Finnish nutrition recommendations (20). We speculate that these individuals may be genetically predisposed to low serum 25(OH)D concentration and could benefit from a higher vitamin D intake and personalized dietary advice. It has been shown that genetic factors modify serum 25(OH)D concentration (30-35) as well as response to vitamin D intake (36-41). Genome-wide association studies (GWASs) have identified strong associations between serum 25(OH)D and single nucleotide polymorphisms (SNPs) at or near loci related to vitamin D metabolism (*GC*, GC vitamin D binding protein; *CYP2R1*, cytochrome P450 family 2 subfamily R polypeptide 1; *NADSYN1*, NAD synthetase 1; *DHCR7*, 7-dehydrocholesterol reductase; and *CYP24A1*, cytochrome P450 family 24 subfamily A member 1) (41-51). Also, several novel, less significant loci have recently been identified (43-46, 48-51).

We aimed to personalize vitamin D supplementation recommendations by applying knowledge of genetic factors affecting serum 25(OH)D concentration. We developed a genetic risk score (GRS) for serum 25(OH)D concentration based on a GWAS of serum 25(OH)D in a large Finnish biobank cohort. This population-matched GRS was applied to tailor vitamin D supplementation recommendations for the participants of a longitudinal

77 Digital Health Revolution (DHR) Study. Due to the repeated serum 25(OH)D measurements
78 and comprehensive lifestyle data collected over 16 months, the DHR Study provided an
79 excellent opportunity to study changes in individual serum 25(OH)D concentrations and to
80 monitor how genotype-based personalized vitamin D dosing affected 25(OH)D
81 concentrations.

METHODS

Ethical permits

The DHR Study and the H2011 Survey were conducted according to the guidelines of the Declaration of Helsinki and approved by the Coordinating Ethics Committee of the Helsinki University Hospital, Helsinki, Finland. Written informed consent was obtained from all the subjects in both studies.

Study populations

The H2011 cohort is a nationally representative sample of Finnish adults. The H2011 Survey (BRIF8901) was conducted from 2011 to 2012 and has previously been described in detail (52). The H2011 Survey data were obtained from THL Biobank (Finnish Institute for Health and Welfare). In this study, we examined those H2011 subjects (n = 3,826) from whom genotype data was available (**Supplemental Table 1**).

The DHR Study was conducted at the Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki, Finland, between September 2015 and January 2017. We recruited 107 volunteers (aged from 25 to 59) of European descent from the clientele of a private occupational healthcare service provider (Mehiläinen Töölö, Helsinki, Finland). Pregnant women and individuals with severe chronic diseases (e.g., cardiovascular disease, diabetes, or cancer) were excluded, but overweight and obese subjects and individuals with risk factors for chronic diseases were allowed to participate. Participants were required to

have sufficient computer skills as well as access to the Internet via a smartphone, which was compatible with a smartwatch used in the study. Participants were expected to have sufficient knowledge of English to be able to understand simple messages and to use health and wellness applications. All the participants lived within the Helsinki Metropolitan area. The descriptive characteristics of 96 participants who completed the study are shown in **Table 1**.

The DHR Study included five study visits approximately once every four months. A health check, including measurements of body weight, height, waist and hip circumferences, blood pressure[RS4], and pulse, was performed at every visit. Participants donated blood, urine, saliva, and fecal samples five times for clinical chemistry, genomics, proteomics, metabolomics, and gut microbiome analyses. Fasting blood glucose was measured using… Total cholesterol and HDL cholesterol were measured using… Throughout the study, we collected longitudinal data using a comprehensive questionnaire, fitness tests, digital monitoring of physical activity and sleep, and digital grocery shopping histories. Actionable personal health data were returned to participants via a health dashboard and web applications starting at study visit 2. These data were the basis for tailored health and wellness advice and coaching provided by two personal trainers from visit 3 onward.

Measurement of serum 25(OH)D concentrations

In the H2011 cohort, serum 25(OH)D concentrations were measured using a chemiluminescent immunoassay (Architect ci8200; Abbott Laboratories, Abbott Park, IL, USA) at the Laboratory of Biochemistry at THL (Helsinki, Finland) (20). The measurements

were standardized according to the Vitamin D Standardization Program (VDSP) at the University College Cork (Cork, Ireland), as previously described (53).

In the DHR Study, serum 25(OH)D concentrations were measured at the diagnostic laboratory of Mehiläinen Töölö (Helsinki, Finland) after every study visit. Blood samples were collected after an overnight fast and processed to serum. Serum 25(OH)D concentrations were measured using an automated two-step competitive immunoassay (Tosoh Bioscience 25OH vitamin D assay; Tosoh Bioscience, CA, USA). The assay was calibrated against NIST SRM 2972 reference material. The inter-assay CV was 1.5-5.0% in the range 33-131 nmol/L, and the intra-assay CV was 2.2% in the range 33-150 nmol/L.

Serum 25(OH)D concentrations are reported in nmol/L and can be converted to ng/mL by dividing by 2.5. We defined vitamin D deficiency as serum 25(OH)D concentration <50 nmol/L, since serum concentration of 50 nmol/L has been considered sufficient (24, 29).

Assessment of vitamin D intake

According to the Finnish nutrition recommendations, daily consumption of fortified fluid milk products and fortified fat spreads, and consumption of fish at least twice per week is adequate to reach sufficient vitamin D intake (54). A semi-quantitative food frequency questionnaire (FFQ) was used to assess diet in the H2011 cohort (52, 55, 56). The average food consumption and intake of nutrients per day were calculated based on the continuously updated National Finnish Food Composition Database (Fineli) using Finessi, software developed at THL (57). Based on the average daily food consumption, we derived the

following dichotomous (yes/no) food indexes for the H2011 cohort: weekly consumption of fish and fish products ≥300 g, daily consumption of milk (including milk used in cooking) ≥500 g, and daily consumption of fat spreads ≥10 g (20). Thus, meeting all the three food index criteria sums up to approximately 10 μg daily vitamin D intake. FFQ data on dietary supplements containing vitamin D was used to categorize the H2011 subjects into those taking vitamin D ≥10 μg/d and those taking <10 μg/d.

Before every study visit, the DHR Study participants filled out a 51-item non-quantitative FFQ developed for the study. The FFQ included nine frequency categories ranging from never or less than once per month to six times a day or more often. Similarly to the food indexes calculated for the H2011 cohort, we generated dichotomous food indexes for the DHR Study cohort: consuming fish, shellfish, and foods containing them at least twice per week; drinking fortified liquid milk products, including soya, oat, and nut milk, at least twice per day; and consuming margarine or plant sterol or stanol margarine at least twice per day. Data on the frequency of using vitamin D supplements (daily or almost daily, occasionally or periodically, never) was obtained from the main questionnaire.

Genotyping

The H2011 cohort was genotyped in several batches using different versions of Illumina genotyping arrays (Illumina HumanCoreExome-24-v1-1_A, Illumina Human610K, and Illumina Human610-Quadv1_B; Illumina Inc, San Diego, CA, USA). The genotyping data was prephased and imputed using the Sequencing Initiative Suomi (SISu) (58) reference panel

2 (with 2,690 whole-genome sequenced and 5,092 whole-exome sequenced Finnish genomes) and Impute2. After quality control (Hardy-Weinberg equilibrium (HWE) $P < 0.001$, minor allele frequency <1.0%, imputation info <0.8), the imputed GWAS data set contained almost 7.7 million SNPs from 3,339 samples.

Genotyping of the DHR Study cohort was performed at the FIMM Technology Centre (HiLIFE, University of Helsinki, Finland) using InfiniumCoreExome-24 v1.0 DNA Analysis Kit, iScan system, standard reagents, and protocols provided by Illumina (Illumina Inc, San Diego, CA, USA). SNP rs4588 was genotyped also using the Agena MassARRAY system and the iPLEX Gold assay (Agena Bioscience, San Diego, CA, USA). The genotyping results were identical between the two platforms. Both rs4588 and rs10741657 were in HWE ($P > 0.05$).

Generation of the genetic risk score (GRS)

To provide personalized vitamin D supplementation recommendations, we developed a GRS for serum 25(OH)D concentration. We started by evaluating the published GRSs, usually including two to four SNPs from two to three genomic loci (38, 42, 59, 60). These GRSs were not optimal for our study for several reasons. First, the source GWASs have been performed in mixed populations, and some of the GRSs include several highly correlated SNPs from a single locus. Second, as SNP allele frequencies vary widely across populations, we wanted to ensure that the GRS matches the genetic characteristics of the study population. Third, the study population in Finland is affected by a low amount of sunlight in the wintertime and the

consumption of vitamin D fortified foods, which may impact the genetic loci affecting vitamin D metabolism. Thus, in order to develop a GRS optimized for the Finnish population, we performed a GWAS of serum 25(OH)D concentration in the H2011 cohort. Based on the GWAS results and the current literature, we generated a population-matched two-SNP GRS consisting of one SNP from each of the genome-wide significant loci, i.e., *GC* and *CYP2R1* (**Figure 1**). We calculated the GRS (range from 0 to 4) as the sum of the number of risk alleles for the SNPs rs4588 (A allele, minor allele) and rs10741657 (G allele, major allele). rs10741657 is located in the promoter region of the *CYP2R1* gene, whereas rs4588 in the *GC* gene results in the Thr436Lys amino acid change in the Vitamin D-binding protein. Both SNPs have previously been associated with serum 25(OH)D concentration in individuals of European ancestry (38, 42, 46, 59, 60).

Personalized vitamin D supplementation and dietary guidance in the DHR Study

The DHR Study participants received data on their serum 25(OH)D concentrations for the first time after study visit 2. At that point, they were able to compare their results from the first and second visits to the reference values. A study physician interpreted the results of 25(OH)D measurements, along with several other laboratory tests, to the participants in a group meeting. The participants were able to discuss their results with the physician, but no vitamin D supplementation recommendations were given at this point. Personalized vitamin D supplementation and dietary guidance was launched at study visit 4 in August 2016. The participants received tailored vitamin D supplementation recommendations based on their GRS, serum 25(OH)D concentration, use of vitamin D supplements, and dietary choices

(**Table 2, Figure 2**). These data and comparison to other participants' results, as well as supplementation recommendations and dietary advice, were communicated to the participants via the health dashboard and a web application developed during the study. The GRS was also presented to the participants in a group meeting soon after study visit 4. In November 2016, after a follow-up of three and a half months, serum 25(OH)D concentrations were remeasured and lifestyle data collected. Data were once more returned to the participants, and experiences of receiving the GRS were queried. Updated recommendations were provided to those who still had their serum 25(OH)D concentration <50 nmol/L.

Statistical analyses

Data are presented as mean ± SD or range for continuous variables and frequencies and percentages for categorical variables. The DHR Study participants with three or four risk alleles were combined for analyses since only one individual had four risk alleles. Statistical analyses were performed using R version 3.5.1 (2018-07-02), x86_64-apple-darwin15.6.0 (61), SPSS Statistics version 25 (IBM), and PLINK version 2 (62, 63). A nominal *P*-value < 0.05 was considered statistically significant. For the GWAS, a commonly used genome-wide significance threshold of 5×10^{-8} was applied (64).

The GWAS of serum 25(OH)D concentration in the H2011 cohort (n = 3,339) was performed using multiple linear regression. Serum 25(OH)D concentration was log-transformed, and age, sex, BMI, sampling month, vitamin D from supplements, and the food indexes for milk, fish and fish products, and fat spreads were included as covariates. Age and BMI were

included as continuous variables, sampling month as a categorical variable (five categories), and the other covariates as dichotomous variables.

Minor allele and GRS frequencies were compared between the H2011 and DHR Study cohorts using the chi-square test. Association between the GRS and serum 25(OH)D concentration was tested using the One-Way ANOVA and multiple linear regression analysis adjusted for age, sex, BMI, sampling month (H2011), vitamin D supplement use, and the food indexes. The Cochran-Armitage trend test was used to examine a linear trend between the GRS and vitamin D deficiency.

The linear mixed-effect model was used to analyze changes in serum 25(OH)D concentrations between the DHR Study visits. The subject identifier and BMI were modeled as random effects and age and sex as fixed effects. The repeated measures analysis of variance was used to perform trend analysis over the DHR Study visits including time (study visit) and GRS interaction effect. The McNemar test was used to compare the prevalence of vitamin D deficiency and the consumption of vitamin D supplements and vitamin D-rich foods between the study visits. Fisher’s Exact Test was used to examine if the participants experienced receiving the GRS data differently depending on their GRS.

RESULTS

Development and validation of a population-matched GRS

The GWAS in the H2011 cohort (*n* = 3,339) showed that two genetic loci had a genome-wide significant contribution to serum 25(OH)D concentration in the Finnish population (Figure 1A, B). Both of these loci represent the well-known vitamin D genes *GC* and *CYP2R1*. We developed a two-SNP GRS for serum 25(OH)D including one SNP from each of these two loci: rs4588 ($P = 1.2 \times 10^{-22}$; $\beta = -3.7$ nmol/L) from the *GC* gene and rs10741657 ($P = 4.9 \times 10^{-12}$; $\beta = 2.2$ nmol/L) from the *CYP2R1* gene (**Supplemental Table 2**, Figure 1C). We calculated the GRS (range from 0 to 4) as the sum of the number of risk alleles for rs4588 (A allele, minor allele) and rs10741657 (G allele, major allele). The resulting five GRS groups did not significantly differ in frequency between the H2011 and DHR Study cohorts ($P = 0.16$) (**Supplemental Table 3**). Moreover, there were no differences in the minor allele frequencies of rs4588 (20.1% in H2011 vs. 21.4% in DHR; $P = 0.68$) and rs10741657 (42.1% in H2011 vs. 39.1% in DHR; $P = 0.41$).

Supplemental Figure 1 shows serum 25(OH)D concentrations in the H2011 and DHR Study cohorts stratified by the GRS. The GRS was associated with serum 25(OH)D concentration in both cohorts after adjusting for age, sex, BMI, sampling month (H2011), and consumption of vitamin D supplements, fish, milk, and fat spreads (**Table 3** and Supplemental Table 2). The association was highly significant ($P = 5.4 \times 10^{-32}$) in the H2011 cohort, and one GRS unit corresponded to a shift of 2.8 nmol/L in serum 25(OH)D concentration. In the DHR Study cohort, we found an association between the GRS and serum 25(OH)D concentration at study

visits 1 (P = 0.05), 3 (P = 0.04), and 4 (P = 0.01). Furthermore, an increasing GRS was strongly associated with vitamin D deficiency (serum 25(OH)D <50 nmol/L) in the H2011 cohort ($P = 8.7 \times 10^{-5}$), and this was repeated in the DHR Study cohort at study visits 1 (P = 0.0097) and 2 (P = 0.049).

Personalized vitamin D supplementation and dietary guidance in the DHR Study

During the study, the prevalence of vitamin D deficiency decreased from 31.3% to 15.7% (P = 0.006) with the mean serum 25(OH)D concentration increasing from 61.0 (± 23.5) nmol/L to 68.5 (± 19.2) nmol/L (P = 0.001 adjusted for age, sex, and BMI) (**Figure 3A**, **B**). The most prominent decrease in the prevalence of vitamin D deficiency, from 26.3% to 15.7% (P = 0.12), was observed following the personalized vitamin D supplementation and dietary guidance between study visits 4 and 5 (**Supplemental Table 4**, **Supplemental Figure 2A**). Between these data points, the mean serum 25(OH)D concentration increased from 64.4 (± 20.9) nmol/L to 68.5 (± 19.2) nmol/L (P = 0.006 adjusted for age, sex, and BMI).

The positive effect of the guidance was notable when examining the results separately within each GRS group (Figure 3C, D). The mean serum 25(OH)D concentration increased in all the GRS groups between study visits 1 through 3 and decreased between visits 3 and 4 (P = 0.0004 for time effect), compatible with the concept that all the participants were subject to the same overall health guidance (Figure 3C, Table 3). This profile of changes was similar in all the GRS groups (P = 0.41 for time and GRS interaction effect). Following the communication of the GRS and guidance for personalized vitamin D supplementation and

diet, the mean serum 25(OH)D concentration increased in the participants with three or four risk alleles but decreased in the participants without risk alleles. As a result, the difference between these genetic subgroups decreased significantly from 20.7 nmol/L to 8.0 nmol/L (P = 0.0063). Moreover, the prevalence of vitamin D deficiency tended to decrease from 38.1% to 10.0% among the participants with three or four risk alleles (P = 0.07) (Supplemental Table 4, **Figure 4B**, Supplemental Figure 2B).

We were also interested in monitoring changes in the consumption of vitamin D supplements and vitamin D-rich foods in response to guidance. Between study visits 4 and 5, the percentage of daily or almost daily vitamin D supplement users increased from 32.6% to 60.2% ($P = 6.5 \times 10^{-6}$) (**Supplemental Table 5**, Figure 4C, **Supplemental Figures 3A** and **4A**). Stratifying by the GRS showed that the increase was significant in the participants with one (P = 0.01), two (P = 0.02), and three or four (P = 0.001) risk alleles but not in the participants without risk alleles (P = 0.99) (Figure 4C, Supplemental Figures 3B and 4B). We did not observe any significant changes in the consumption of vitamin D-rich foods between study visits 4 and 5. However, a slightly higher proportion of participants consumed fish and milk at study visit 5 compared to visit 4 (Supplemental Table 5, Figure 4D–F, **Supplemental Figure 5**). We emphasized the importance of consuming vitamin D-rich foods to participants with serum 25(OH)D concentration <50 nmol/L, but we did not observe any significant changes in the consumption of fish, milk, or fat spreads (data not shown). Neither did we observe any significant changes in diet after stratifying by the GRS (Supplemental Table 5, Figure 4D-F).

The participants' views of receiving the GRS data are summarized in **Supplemental Table 6**. Overall, 52.8% of the participants strongly agreed that receiving data on the genetic risk factors affecting serum 25(OH)D concentration was important regarding their health. Of those with three or four risk alleles, 80.0% strongly agreed on the GRS data being important regarding their health. Most of the participants thought that receiving the GRS did not worry (83.2%) or stress (83.1%) them.

DISCUSSION

We report here the development, validation, and implementation of a GRS for serum 25(OH)D concentration to personalize vitamin D supplementation. With the electronic return of the GRS, serum 25(OH)D concentrations, and personalized supplementation as well as dietary advice directly to the DHR Study participants, the daily use of vitamin D supplements was rationalized, resulting in an increased mean serum 25(OH)D concentration, decreased inter-individual variation, and a lower prevalence of vitamin D deficiency (serum 25(OH)D <50 nmol/L). The positive effect was most evident in the participants with multiple risk alleles predisposing to low serum 25(OH) concentration, as one would expect. Therefore, our study demonstrates a proof of concept in which the return of personal molecular data was successfully implemented, along with personalizing vitamin D supplementation.

Up to one-third of inter-individual variability in serum 25(OH)D concentrations can be explained by lifestyle and other known factors (32, 65, 66). Furthermore, in population-based studies, genetic variation has been reported to account for up to 7.5% of the variance in serum 25(OH)D (42, 43, 46, 50, 67), with an oligogenic (34, 67) or moderately polygenic architecture (50). There are, however, differences between populations in terms of the genetic variants affecting serum 25(OH)D concentration (47, 59). Here, we first explored genetic factors that are most important in determining serum 25(OH)D in the Finnish population by carrying out a GWAS of serum 25(OH)D concentration in the H2011 cohort of healthy Finnish adults. The GWAS showed that there were only two genetic loci, i.e., *GC* and *CYP2R1*, which independently modify serum 25(OH)D concentration in the Finnish population in a genome-wide significant manner. The associations of the individual SNPs in

these loci with serum 25(OH)D concentration are similar to those of many previous studies conducted in populations of European ancestry (38, 42, 46, 47, 50, 51, 60). We then developed a simple GRS for serum 25(OH)D concentration with one previously highlighted SNP (rs4588 and rs10741657) from each of the genome-wide significant loci. The more risk alleles an individual carried (i.e., the higher the GRS), the more prone he/she was to have low serum 25(OH)D concentration. In the H2011 cohort, one GRS unit corresponded to a shift of 2.8 nmol/L in serum 25(OH)D, which is not only statistically significant but also clinically meaningful, especially when comparing individuals without any risk alleles and those with four risk alleles.

The prevalence of vitamin D deficiency was higher among the DHR Study participants (26.3%) in August 2016 than in the H2011 subjects (6.5%) sampled from August to December 2011. The observed difference may be due to the different 25(OH)D measurement methods applied and the standardization of the H2011 measurements according to the VDSP (53). Following the guidance provided in the DHR Study, the mean serum 25(OH)D concentration increased, and the prevalence of vitamin D deficiency decreased from 26.3% to 15.7% during the 3.5-month follow-up. Notably, the mean serum 25(OH)D concentration increased in the combined group of participants with the highest GRSs (i.e., three or four risk alleles) and decreased among the participants without any risk alleles. Importantly, the association between the GRS and serum 25(OH)D disappeared after the dietary and supplementation advice, as one would expect.

It has previously been shown that there are significant seasonal variations in serum 25(OH)D concentrations in the Finnish population and that the concentrations decrease rapidly after the

summer months (68). We[RS5] monitored changes in serum 25(OH)D concentrations in the DHR Study cohort from August to November. Vitamin D cannot be synthesized in the skin in Northern latitudes during the winter months (from October to March) due to the lack of UVB radiation (1). Therefore, the positive changes observed in the mean serum 25(OH)D concentrations and the prevalence of vitamin D deficiency were not due to sun exposure.

Our results indicate that individuals with multiple risk alleles predisposing to low serum 25(OH)D require higher than the currently recommended intake of vitamin D (10 μg/d in the Nordic countries (29)) to achieve and maintain adequate serum 25(OH)D concentration. These individuals could benefit from vitamin D supplementation, especially during the dark time of the year. Similar observations have been reported in previous studies (38, 39, 69). Moreover, as the risk of harmful effects is low up to the dosing of 100 μg per day (23), even higher supplementation levels could be safe (16).

Following the guidance, we observed a significant increase in the number of daily or almost daily vitamin D supplement users, especially among the participants with risk alleles. However, we did not observe significant changes in the consumption of vitamin D-rich foods despite the recommendations and room for improvement. It may be that the participants felt it easier to start taking supplements than changing their diet. It could also be that the participants found dietary advice too general, or that the FFQ was not detailed enough to measure changes that may have taken place.

There are few previous studies to suggest genotype-based dietary advice to motivate behavior change (70, 71). Our study on directly communicating and advising study participants based on nutrigenetics information shows that personalized guidance was successful as objectively

measured by serum 25(OH)D concentrations. Furthermore, especially those participants with the highest risk for low vitamin D status thought that the GRS information was important. Our results show that returning the GRS for serum 25(OH)D does not carry a major risk and can help to tailor personalized vitamin D supplementation and to improve vitamin D status. This could, in return, decrease the risk of osteoporosis and potentially other diseases.

Testing[RS6] of serum 25(OH)D concentration in the general population and subsequent supplementation with vitamin D have been under debate (26, 28, 72, 73). Many researchers have been concerned that testing of serum 25(OH)D and supplementation increase the burden and costs of the healthcare system (28, 73). For example, Pilz et al. concluded that measuring serum 25(OH)D concentration should not be used as a population-wide screening tool but applied only in selected individuals at high risk of vitamin D deficiency (28). Our proof of concept study provides several insights into this debate. First, we show that returning data on the genetic risk and serum 25(OH)D concentration directly to participants and guiding them regarding diet and vitamin D supplementation can be successfully accomplished via a web application. This should alleviate the costs and burden for healthcare, particularly in the context of repeated tests. Of course, proper consultation and personal advice should still be provided as a backup. Second, our data suggest that the knowledge on the genetic propensity for low serum 25(OH)D, combined with dietary and supplementation data, could help to identify those individuals who most likely benefit from screening serum 25(OH)D. A recent study by Hatchell et al. also suggested that polygenic risk scores could be used as predictive tools for determining serum 25(OH)D concentrations and personalized vitamin D supplementation (74), but we think that the combination of genetics, environmental, and dietary factors will be even more powerful. Third, genetic testing, measuring serum 25(OH)D,

403 and data on dietary habits could help to target monitoring and supplementation to individuals
404 who most likely need them as well as reduce repeated testing and unnecessary or excessive
405 supplementation for those who do well without them.

406 This study was designed to test procedures as well as attitudes of people and, therefore, needs
407 to be validated in external datasets. Our study had a longitudinal design that originated from
408 the DHR Study, including five study visits during 16 months and examining a cohort that is
409 rather homogeneous geographically, ethnically, genetically, and in terms of diet and lifestyle.
410 We think that these advantages partly compensate for the rather small size of the DHR Study
411 cohort. Furthermore, the use of the much bigger, independent, and population-matched H2011
412 cohort was important. There was also comprehensive data available on most of the known
413 risk factors for low serum 25(OH)D from both cohorts.

414 In conclusion, we show that the GRS we developed helped to identify individuals who are
415 genetically predisposed to low serum 25(OH)D concentration and, therefore, would benefit
416 most from monitoring serum 25(OH)D as well as from a higher vitamin D intake. Our results
417 apply to the Finnish population and environmental conditions. However, we think that the
418 model could be modified to fit to other populations, geographical regions, and countries. We
419 thus propose that the direct return of data on individual genetic risk for low vitamin D status,
420 serum 25(OH)D concentration, and lifestyle via a web or smartphone application could be
421 used to guide personalized vitamin D supplementation and promote individualized health.

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TABLES

TABLE 1 Descriptive characteristics of the DHR Study participants at study visit 1

	Female	**Male**	**All**
n	66	30	96
Age, years	41.1 (25-59)	40.5 (26-59)	40.9 (25-59)
Education >12 years	83.3 (55)	93.3 (28)	86.5 (83)
Married or cohabiting	62.1 (41)	76.7 (23)	66.7 (64)
BMI, kg/m^2	24.5 (4.8)	25.9 (4.3)	24.9 (4.6)
Systolic blood pressure, mmHg	120.6 (14.3)	134.1 (17.3)	124.8 (16.5)
Diastolic blood pressure, mmHg	78.3 (9.2)	82.0 (10.5)	79.5 (9.7)
Fasting blood glucose, mmol/L	5.3 (0.6)	5.6 (0.5)	5.4 (0.6)
Cholesterol/HDL cholesterol ratio	2.7 (1.0)	3.3 (1.0)	2.9 (1.0)
Serum 25(OH)D, nmol/L	62.0 (24.5)	58.8 (21.4)	61.0 (23.5)
Serum 25(OH)D <50 nmol/L	30.3 (20)	33.3 (10)	31.3 (30)
Physical training ≥3 times/wk	53.0 (35)	63.3 (19)	56.3 (54)
Current smoker[1]	17.2 (11)[2]	16.7 (5)	17.0 (16)[3]
Consumption of alcohol (ethanol), g/wk	44.7 (41.7)	71.6 (68.6)	53.1 (52.8)

Values are means (range), means (± SD) or percent (*n*).

[1]Participants who reported regular smoking or had smoked during the previous month were defined as current smokers.

[2]*n* = 64.

[3]*n* = 94.

DHR, Digital Health Revolution; 25(OH)D, 25-hydroxyvitamin D.

TABLE 2 Vitamin D supplementation and diet recommendations given to the DHR Study participants after study visit 4

	Serum 25(OH)D concentration at study visit 3 (after the winter season)[1]		
	<50 nmol/L	**50-125 nmol/L**	**>125 nmol/L**
Vitamin D supplementation recommendations	GRS[2] 0: 10-20 µg/d[3] GRS[2] 1-2: 20-30 µg/d[3] GRS[2] 3-4: 50 µg/d[3]	No need for changes compared to the period between study visits 2 and 3.	Not recommended.
Diet recommendations	Eat fish two to three times per week and consume fortified liquid milk products and fat spreads daily. Special focus on the consumption of vitamin D-rich foods.	Eat fish two to three times per week and consume fortified liquid milk products and fat spreads daily.	Eat fish two to three times per week and consume fortified liquid milk products and fat spreads daily.

[1]Recommendations were based on serum 25(OH)D concentrations at study visit 3 describing vitamin D status during the dark time of the year.

[2]The GRS (range from 0 to 4) was calculated as the sum of the number of risk alleles for the SNPs rs10741657 (G allele) in the *CYP2R1* gene and rs4588 (A allele) in the *GC* gene.

[3]The lower dose was recommended if fish was eaten at least twice per week and fortified liquid milk products and fat spreads were consumed daily. If a bigger dose of vitamin D was already in use, it was recommended to be continued.

CYP2R1, cytochrome P450 family 2 subfamily R polypeptide 1; DHR, Digital Health Revolution; *GC*, GC vitamin D binding protein; GRS, genetic risk score; SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.

TABLE 3 Mean serum 25(OH)D concentrations and the association of the GRS with serum 25(OH)D in the DHR Study cohort

Visit	Sampling month and year	Serum 25(OH)D, nmol/L (± SD)					P[2]	P_{adj}[3]	Effect size[3]
		All (n = 96)[4]	GRS 0 (n = 10)[4]	GRS 1 (n = 36)[4]	GRS 2 (n = 29)[4]	GRS 3+4[1] (n = 21)[4]			
1	October 2015	61.0 (23.5)	66.5 (18.1)	63.9 (28.4)	59.6 (22.3)	55.2 (17.4)	0.49	0.05	-4.9
2	January 2016	65.5 (23.6)	74.4 (16.0)	65.9 (24.4)	66.5 (25.7)	59.1 (21.7)	0.39	0.08	-4.3
3	April 2016	66.8 (22.9)	79.8 (15.9)	68.0 (22.9)	65.3 (25.9)	60.7 (19.4)	0.18	0.04	-4.7
4	August 2016	64.4 (20.9)	78.6 (21.5)	64.6 (20.4)	64.1 (20.5)	57.9 (20.1)	0.08	0.01	-6.0
Personalized guidance									
5	November 2016	68.5 (19.2)	74.9 (19.7)	68.4 (17.7)	67.8 (23.2)	66.9 (16.0)	0.76	0.33	-2.2

The GRS (range from 0 to 4) was calculated as the sum of the number of risk alleles for the SNPs rs10741657 (G allele) in the *CYP2R1* gene and rs4588 (A allele) in the *GC* gene.

[1]Participants with three or four risk alleles were combined for analyses since only one participant had four risk alleles.

[2]Unadjusted *P*-value for the association of the GRS with serum 25(OH)D concentration calculated using the One-Way ANOVA.

[3]*P*-value and unstandardized β coefficient (change in serum 25(OH)D (nmol/L) per one GRS unit) for the association of the GRS with serum 25(OH)D concentration calculated using multiple linear regression and adjusted for age, sex, BMI, vitamin D supplement use (daily, occasionally, never), and the food indexes for milk, fish, and fat spreads.

[4]Number of participants at study visit 1.

CYP2R1, cytochrome P450 family 2 subfamily R polypeptide 1; DHR, Digital Health Revolution; *GC*, GC vitamin D binding protein; GRS, genetic risk score; SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.

FIGURE LEGENDS

FIGURE [RS7]**1** GWAS results for serum 25(OH)D concentration in the H2011 cohort (*n* = 3,399) and calculation of the GRS. (A) Manhattan plot of serum 25(OH)D GWAS. The chromosomal positions are on the x-axis and -log10 *P*-values on the y-axis. The horizontal grey line represents the threshold of $P = 5 \times 10^{-8}$ for genome-wide significance. *P*-values were obtained from multiple linear regression adjusted for age, sex, BMI, sampling month, vitamin D from supplements, and consumption of fish, milk, and fat spreads. (B) Quantile-Quantile plot. The x-axis shows the expected -log10 *P*-values and the y-axis the observed -log10 *P*-values. Each SNP is plotted as a black dot, and the grey line indicates the null hypothesis of no true association. (C) The GRS (range from 0 to 4) was calculated as the sum of the number of risk alleles for the SNPs rs4588 (A allele) in the *GC* gene and rs10741657 (G allele) in the *CYP2R1* gene. *CYP2R1*, cytochrome P450 family 2 subfamily R polypeptide 1; *GC*, GC vitamin D binding protein; GRS, genetic risk score; GWAS, genome-wide association study; H2011, Health 2011; SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.

FIGURE 2 Personalized vitamin D supplementation and dietary guidance in the DHR Study. Personalized guidance was based on the GRS, serum 25(OH)D concentration, use of vitamin D supplements, and dietary choices. These data, as well as supplementation recommendations and dietary advice, were communicated to the participants via the health dashboard. Direct return of genetic risk data, serum 25(OH)D concentration, and lifestyle data via a web or smartphone application could be used to guide personalized vitamin D supplementation. DB, database; DHR, Digital Health Revolution; FFQ, food frequency questionnaire; GRS, genetic risk score; S25(OH)D, serum 25-hydroxyvitamin D.

FIGURE 3 Mean serum 25(OH)D concentrations and the prevalence of vitamin D deficiency (defined as serum 25(OH)D <50 nmol/L) in the DHR Study cohort. (A) Mean serum 25(OH)D concentrations and (B) the prevalence of vitamin D deficiency in all the participants. (C) Mean serum 25(OH)D concentrations and (D) the prevalence of vitamin D deficiency stratified by the GRS. Personalized vitamin D supplementation and dietary guidance were implemented soon after study visit 4. The dots indicate the mean, the error bars the SEM, and the grey zone 95% confidence interval. The GRS (range from 0 to 4) was calculated as the sum of the number of risk alleles for the SNPs rs4588 (A allele) in the *GC* gene and rs10741657 (G allele) in the *CYP2R1* gene. The number of participants stratified by the GRS refers to visit 1. *CYP2R1*, cytochrome P450 family 2 subfamily R polypeptide 1; DHR, Digital Health Revolution; *GC*, GC vitamin D binding protein; GRS, genetic risk score; S25(OH)D, serum 25-hydroxyvitamin D; SNP, single nucleotide polymorphism.

FIGURE[RS8] **4** Vitamin D status of the DHR Study participants and the consumption of vitamin D supplements and vitamin D-rich foods before (study visit 4) and after (study visit 5) the personalized vitamin D supplementation and dietary guidance. Data are shown for all the participants and stratified by the GRS. (A) Mean serum 25(OH)D concentrations. The respective GRS line is colored, and the lines for the other GRS groups are shown in grey. (B) The prevalence of vitamin D deficiency (i.e., serum 25(OH)D <50 nmol/L). (C) The percentage of daily or almost daily vitamin D supplement users. The percentage of participants consuming (D) fish at least twice per week, (E) fortified liquid milk products at least twice per day, and (F) margarine or plant sterol or stanol margarine at least twice per day. The GRS (range from 0 to 4) was calculated as the sum of the number of risk alleles for the SNPs rs4588 (A allele) in the *GC* gene and rs10741657 (G allele) in the *CYP2R1* gene.

Bars are colored if the change between the visits was bigger than 5%. Significant[RS9] changes (*P*-value < 0.05) are marked with stars. The *P*-values were obtained from (A) the linear mixed-effect model (subject identifier and BMI as random effects and age and sex as fixed effects) and (B-F) the McNemar test. *CYP2R1*, cytochrome P450 family 2 subfamily R polypeptide 1; DHR, Digital Health Revolution; *GC*, GC vitamin D binding protein; GRS, genetic risk score; S25(OH)D, serum 25-hydroxyvitamin D; SNP, single nucleotide polymorphism.