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Sallinen, Riitta J.

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

Riitta J Sallinen,[1,2] Olga Dethlefsen,[3] Sanni Ruotsalainen,[1] Robert D Mills,[1] Timo A Miettinen,[1] Tuija E Jääskeläinen,[4] Annamari Lundqvist,[4] Eero Kyllönen,[5] Heikki Kröger,[6,7] Jaro I Karppinen,[8,9] Christel Lamberg-Allardt,[10] Heli Viljakainen,[10,11] Mari A Kaunisto,[1] and Olli Kallioniemi[1,2] on behalf of the Digital Health Revolution Project

[1]Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki, Helsinki, Finland; [2]Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden; [3]National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Stockholm University, Stockholm, Sweden; [4]Finnish Institute for Health and Welfare, Department of Public Health Solutions, Helsinki, Finland; [5]Physical and Rehabilitation Medicine Division, Oulu University Hospital, Oulu, Finland; [6]Department of Orthopaedics, Traumatology and Handsurgery, Kuopio University Hospital, Kuopio, Finland; [7]Kuopio Musculoskeletal Research Unit, Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland; [8]Medical Research Center Oulu, University of Oulu and Oulu University Hospital, Oulu, Finland; [9]Finnish Institute of Occupational Health, Oulu, Finland; [10]Department of Food and Nutrition, University of Helsinki, Helsinki, Finland; and [11]Folkhälsan Research Center, Helsinki, Finland

ABSTRACT

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

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

Methods: We performed a genome-wide association study of serum 25(OH)D concentration in the Finnish Health 2011 cohort ($n = 3339$) using linear regression and applied the results to develop a population-matched genetic risk score (GRS) for serum 25(OH)D. This GRS was used to tailor vitamin D supplementation for 96 participants of a longitudinal Digital Health Revolution (DHR) Study. The GRS, serum 25(OH)D concentrations, and personalized supplementation and dietary advice were electronically returned to participants. Serum 25(OH)D concentrations were assessed using immunoassays and vitamin D intake using FFQs. In data analyses, cross-sectional and repeated-measures statistical tests and models were applied as described in detail elsewhere.

Results: GC vitamin D–binding protein and cytochrome P450 family 2 subfamily R polypeptide 1 genes showed genome-wide significant associations with serum 25(OH)D concentration. One single nucleotide polymorphism from each locus (rs4588 and rs10741657) was used to develop the GRS. After returning data to the DHR Study participants, daily vitamin D supplement users increased from 32.6% to 60.2% ($P = 6.5 \times 10^{-6}$) and serum 25(OH)D concentration from 64.4 ± 20.9 nmol/L to 68.5 ± 19.2 nmol/L ($P = 0.006$) between August and November. Notably, the difference in serum 25(OH)D concentrations between participants with no risk alleles and those with 3 or 4 risk alleles decreased from 20.7 nmol/L to 8.0 nmol/L ($P = 0.0063$).

Conclusions: We developed and applied a population-matched GRS to identify individuals genetically predisposed to low serum 25(OH)D concentration. We show how the electronic return of individual genetic risk, serum 25(OH)D concentrations, and factors affecting vitamin D status can be used to tailor vitamin D supplementation. This model could be applied to other populations and countries. *J Nutr* 2021;151:281–292.

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 >1 billion people worldwide (1–3). Vitamin D deficiency can cause rickets and osteomalacia and increase the risk of osteoporosis and bone fractures (4, 5). Low serum 25-hydroxyvitamin D [25(OH)D], the best available biomarker of vitamin D status, has also been associated with

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many other adverse health outcomes, such as cardiovascular disease, cancers, diabetes, autoimmune diseases, infections, and total mortality (4, 6–8). Although mendelian randomization studies provide evidence for causal relations (9–15), randomized controlled trials and their meta-analyses have so far largely failed to prove clinically relevant beneficial effects of vitamin D supplementation on various nonskeletal health outcomes (7, 16–18), except for all-cause mortality in community- and home-dwelling adults, and elderly people (7, 17). However, many studies have been conducted in populations with sufficient vitamin D status, or without optimal dosing of vitamin D, which could contribute to results not supporting the benefits of vitamin D.

Vitamin D deficiency can be prevented and corrected, but it 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, that is, <25–30 nmol/L (22). If we want to have serum 25(OH)D concentration ≥50 nmol/L in the majority of the population, systematic large-scale vitamin D supplementation would be needed (22). However, there is substantial interindividual 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 2 wk of life up to the age of 74 y (29). This intake should maintain a serum 25(OH)D concentration of ∼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 concentrations <50 nmol/L despite the consumption of fish, fortified fluid milk products, and fortified fat spreads as per the Finnish nutrition recommendations (20). We speculate that these individuals could 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) and 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 in the longitudinal Digital Health Revolution (DHR) Study. Due to the repeated serum 25(OH)D measurements and comprehensive lifestyle data collected over 16 mo, the DHR Study provided an excellent opportunity to study changes in individual serum 25(OH)D concentrations and to monitor how genotype-based personalized vitamin D dosing affected 25(OH)D 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 Finnish Institute for Health and Welfare (THL) Biobank. In this study, we examined those H2011 subjects (n = 3826) from whom genotype data were 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 25–59 y) 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 and 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 5 study visits approximately once every 4 mo. A health check, including measurements of body weight, height, waist and hip circumferences, blood pressure, and pulse, was performed at every visit. Participants donated blood, urine, saliva, and fecal samples 5 times for clinical chemistry, genomics, proteomics,

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Supplemental Tables 1–6 and Supplemental Figures 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the table of contents at http://academic.oup.com/jn.

Address correspondence to RJS (e-mail: riittajsallinen@gmail.com).

Abbreviations used: *CYP2R1*, cytochrome P450 family 2 subfamily R polypeptide 1; DHR, Digital Health Revolution; 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; S25(OH)D, serum 25-hydroxyvitamin D; SNP, single nucleotide polymorphism; THL, Finnish Institute for Health and Welfare; VDSP, Vitamin D Standardization Program; 25(OH)D, 25-hydroxyvitamin D.

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

	Female	Male	All
n	66	30	96
Age, y	41.1 (25–59)	40.5 (26–59)	40.9 (25–59)
Education >12 y	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
Fasting plasma glucose, mmol/L	5.3 ± 0.6	5.6 ± 0.5	5.4 ± 0.6
Plasma total 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[2]	17.2 (11)[3]	16.7 (5)	17.0 (16)[4]
Consumption of alcohol (ethanol), g/wk	44.7 ± 41.7	71.6 ± 68.6	53.1 ± 52.8

[1] Values are means (range), means ± SD, or % (*n*). DHR, Digital Health Revolution; 25(OH)D, 25-hydroxyvitamin D.
[2] Participants who reported regular smoking or had smoked during the previous month were defined as current smokers.
[3] $n = 64$.
[4] $n = 94$.

metabolomics, and gut microbiome analyses. Fasting plasma glucose (glucose reagents A and B; hexokinase, 981304), total cholesterol (cholesterol reagent; 981812), and HDL cholesterol (HDL cholesterol plus reagents A and B; 981824) were measured using the Konelab Prime 60i Clinical Chemistry Analyzer (ThermoFisher Scientific Oy) at the clinical laboratory of Mehiläinen Töölö. 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 2 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) 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 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 2-step competitive immunoassay (Tosoh Bioscience 25OH vitamin D assay). The assay was calibrated against National Institute of Standards and Technology Standard Reference Material 2972. The interassay 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 nanomoles per liter and can be converted to nanograms per milliliter by dividing by 2.5. We defined vitamin D deficiency as serum 25(OH)D concentration <50 nmol/L, because the 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 semiquantitative FFQ was used to assess diet in the H2011 cohort (52, 55, 56). The average daily food consumption and intake of nutrients 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 3 food index criteria sums to ∼10 μg daily vitamin D intake. FFQ data on dietary supplements containing vitamin D were 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 nonquantitative FFQ developed for the study. The FFQ included 9 frequency categories ranging from never or less than once per month to 6 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) were 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.). The genotyping data were prephased and imputed using the Sequencing Initiative Suomi (SISu) (58) reference panel 2 (with 2690 whole-genome sequenced and 5092 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 3339 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 Inc. SNP rs4588 was genotyped also using the Agena MassARRAY system and the iPLEX Gold assay (Agena Bioscience). The genotyping results were identical between the 2 platforms. Both rs4588 and rs10741657 were in HWE ($P > 0.05$).

Generation of the 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 2–4 SNPs from 2–3 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, because SNP allele frequencies vary widely across populations, we wanted to ensure that the GRS matched the genetic characteristics of the study population.

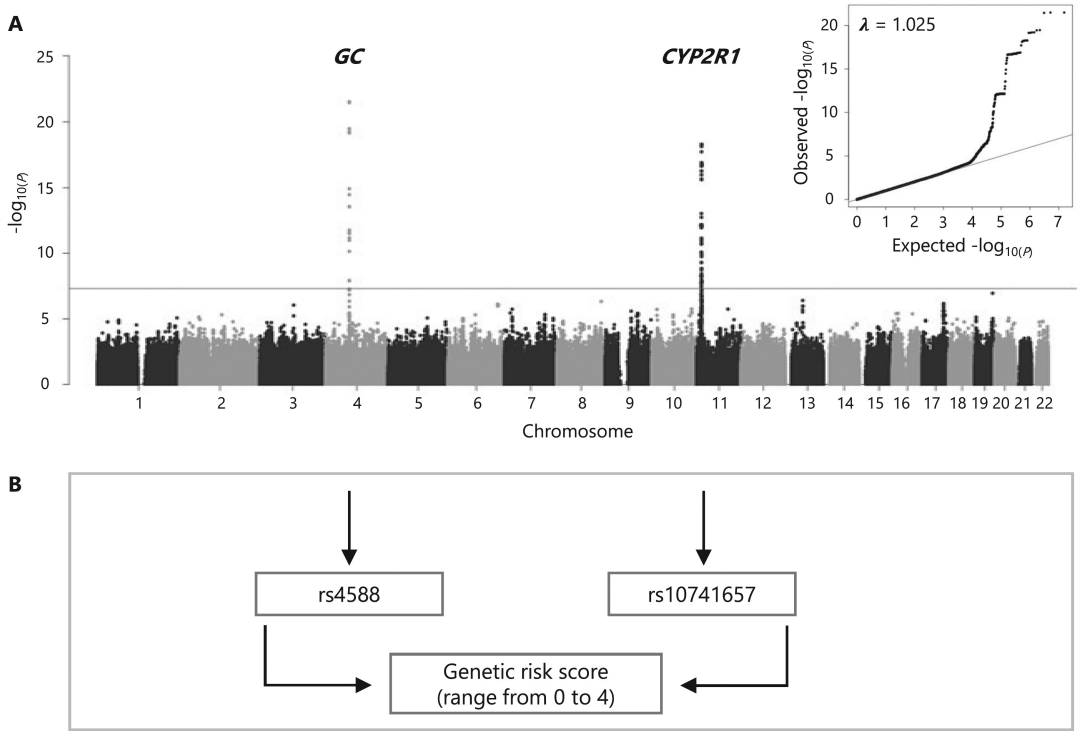

FIGURE 1 GWAS results for serum 25(OH)D concentration in the H2011 cohort ($n = 3399$) and calculation of the GRS. (A) Manhattan plot and quantile-quantile plot of serum 25(OH)D GWAS. In the Manhattan plot, the chromosomal positions are on the *x*-axis and −log10 *P* values on the *y*-axis. The horizontal gray 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. The *x*-axis of the quantile-quantile plot (inset) 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 gray line indicates the null hypothesis of no true association. (B) 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.

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 could impact the genetic loci affecting vitamin D metabolism. Thus, 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 2-SNP GRS consisting of 1 SNP from each of the genome-wide significant loci, that is, 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 with the reference values. A study physician interpreted the results of 25(OH)D measurements, along with several other laboratory tests, for 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 with 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 3.5 mo, serum 25(OH)D concentrations were remeasured and lifestyle data collected. Data were again returned to the participants, and experiences of receiving the GRS were queried. Updated recommendations were provided to those whose serum 25(OH)D concentration remained <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 3 or 4 risk alleles were combined for analyses because only 1 individual had 4 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, the 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 ($n = 3339$) 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

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 with the period between study visits 2 and 3	Not recommended
Diet recommendations	Eat fish 2–3 times/wk and consume fortified liquid milk products and fat spreads daily. Special focus on the consumption of vitamin D–rich foods	Eat fish 2–3 times/wk and consume fortified liquid milk products and fat spreads daily	Eat fish 2–3 times/wk 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. *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.
[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.

as a categorical variable (5 categories), and the other covariates as dichotomous variables.

Minor allele and GRS frequencies were compared between the H2011 and DHR Study cohorts using the χ^2 test. Association between the GRS and serum 25(OH)D concentration was tested using 1-factor 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 ANOVA 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. The Fisher exact test was used to examine if the participants experienced receiving the GRS data differently depending on their GRS.

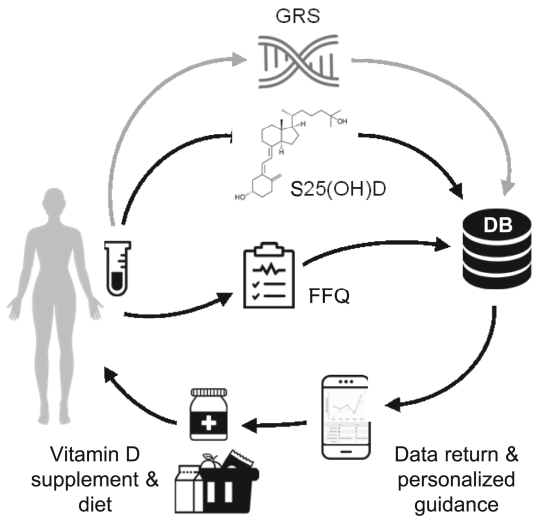

FIGURE 2 Personalized vitamin D supplementation and dietary guidance in the DHR Study. Personalized guidance was based on the GRS, serum 25(OH)D concentration, use of vitamin D supplements, and dietary choices. These data, as well as supplementation recommendations and dietary advice, were communicated 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; GRS, genetic risk score; S25(OH)D, serum 25-hydroxyvitamin D.

Results

Development and validation of a population-matched GRS

The GWAS in the H2011 cohort consisting of individuals with a Finnish ancestry (n = 3339) showed that 2 genetic loci had a genome-wide significant contribution to serum 25(OH)D concentration (Figure 1A). Both loci represent the well-known vitamin D genes *GC* and *CYP2R1*. We developed a 2-SNP GRS for serum 25(OH)D including 1 SNP from each locus: 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 (Figure 1B, **Supplemental Table 2**). 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 5 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 compared with 21.4% in DHR; $P = 0.68$) and rs10741657 (42.1% in H2011 compared with 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 (Supplemental Table 2, Table 3). The association was highly significant ($P = 5.4 \times 10^{-32}$) in the H2011 cohort, and 1 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, a higher 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 ($P = 0.049$).

TABLE 3 Serum 25(OH)D concentrations and the association of the GRS with serum 25(OH)D in the DHR Study cohort[1]

		Serum 25(OH)D, nmol/L							
Visit	Sampling month and year	All ($n = 96$)[3]	GRS 0 ($n = 10$)[3]	GRS 1 ($n = 36$)[3]	GRS 2 ($n = 29$)[3]	GRS 3 + 4[2] ($n = 21$)[3]	P[4]	P_{adj}[5]	Effect size[5]
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

[1]Values are means ± SD. 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. *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.
[2]Participants with 3 or 4 risk alleles were combined for analyses because only 1 participant had 4 risk alleles.
[3]Number of participants at study visit 1.
[4]Unadjusted P value for the association of the GRS with serum 25(OH)D concentration calculated using the 1-factor ANOVA.
[5]P value and unstandardized β coefficient [change in serum 25(OH)D (nmol/L) per 1 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.

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 3**A, 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 Figure 2**A, **Supplemental Table 4**). 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 3 or 4 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 decreased from 38.1% to 10.0% in the participants with 3 or 4 risk alleles, although this failed to reach statistical significance ($P = 0.07$) (Supplemental Figure 2B, Supplemental Table 4, **Figure 4**B).

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 Figures 3A** and **4A**, Figure 4C, **Supplemental Table 5**). Stratifying by the GRS showed that the increase was significant in the participants with 1 ($P = 0.01$), 2 ($P = 0.02$), and 3 or 4 ($P = 0.001$) risk alleles but not in the participants without risk alleles ($P = 0.99$) (Supplemental Figures 3B and 4B, Figure 4C). 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 with visit 4 (Figure 4D–F, **Supplemental Figure 5**, Supplemental Table 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 (Figure 4D–F, Supplemental Table 5).

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 3 or 4 risk alleles, 80.0% strongly agreed on the GRS data being important regarding their health. Most 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 return of the GRS, serum 25(OH)D concentrations, and personalized supplementation and dietary advice electronically to the DHR Study participants, the daily use of vitamin D supplements was rationalized, resulting in an increased mean serum 25(OH)D concentration and a lower prevalence of vitamin D deficiency [serum 25(OH)D <50 nmol/L]. Most importantly, personalized supplementation led to decreased interindividual variability and a statistically significant reduction of differences of serum 25(OH)D concentrations between the genetic risk groups. Thus, with such a personalized approach to supplementation, it was possible to significantly reduce the influence of genetics on the variability of serum 25(OH)D concentrations. 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 the interindividual variability in serum 25(OH)D concentrations can be explained by age, sex, BMI,

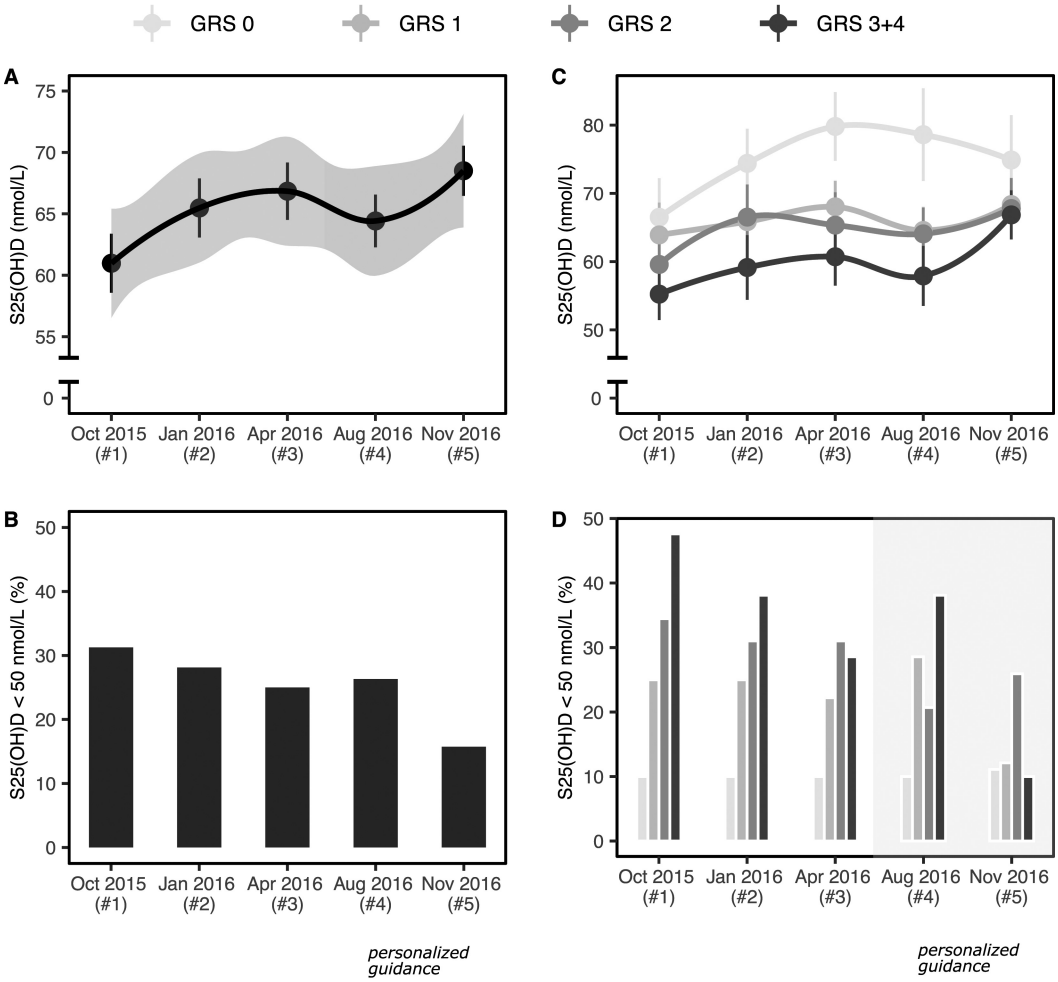

FIGURE 3 Mean serum 25(OH)D concentrations and the prevalence of vitamin D deficiency [defined as serum 25(OH)D <50 nmol/L] in the DHR Study cohort. (A) Mean serum 25(OH)D concentrations and (B) the prevalence of vitamin D deficiency in all the participants. (C) Mean serum 25(OH)D concentrations and (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 gray zone the 95% CI. 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 was 96 at study visits 1–3 ($n_{GRS0} = 10$, $n_{GRS1} = 36$, $n_{GRS2} = 29$, $n_{GRS3+4} = 21$), 95 at visit 4 ($n_{GRS0} = 10$, $n_{GRS1} = 35$, $n_{GRS2} = 29$, $n_{GRS3+4} = 21$), and 89 at visit 5 ($n_{GRS0} = 9$, $n_{GRS1} = 33$, $n_{GRS2} = 27$, $n_{GRS3+4} = 20$). *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.

waist circumference, season, skin exposure to sunlight, geographical latitude, diet, use of vitamin D supplements, physical activity, smoking, and alcohol consumption (32, 65, 66). Furthermore, genetic variation has been reported to account for ≤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 2 genetic loci, namely *GC* and *CYP2R1*, that independently modify serum 25(OH)D concentration in the Finnish population in a genome-wide significant manner. The associations between the individual SNPs in these loci and 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 developed a GRS for serum 25(OH)D concentration with 1 previously highlighted SNP from each locus (rs4588 and rs10741657). 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, 1 GRS unit corresponded to a shift of 2.8 nmol/L in serum 25(OH)D concentration, which is statistically significant and clinically relevant, especially when comparing individuals without any risk alleles and those with 4 risk alleles.

The prevalence of vitamin D deficiency was higher in the DHR Study participants (26.3%) in August 2016 than in the H2011 subjects (6.5%) sampled from August to December 2011. The difference could be due to the different 25(OH)D measurement methods 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 3.5 mo between August (visit 4) and November (visit 5). Notably,

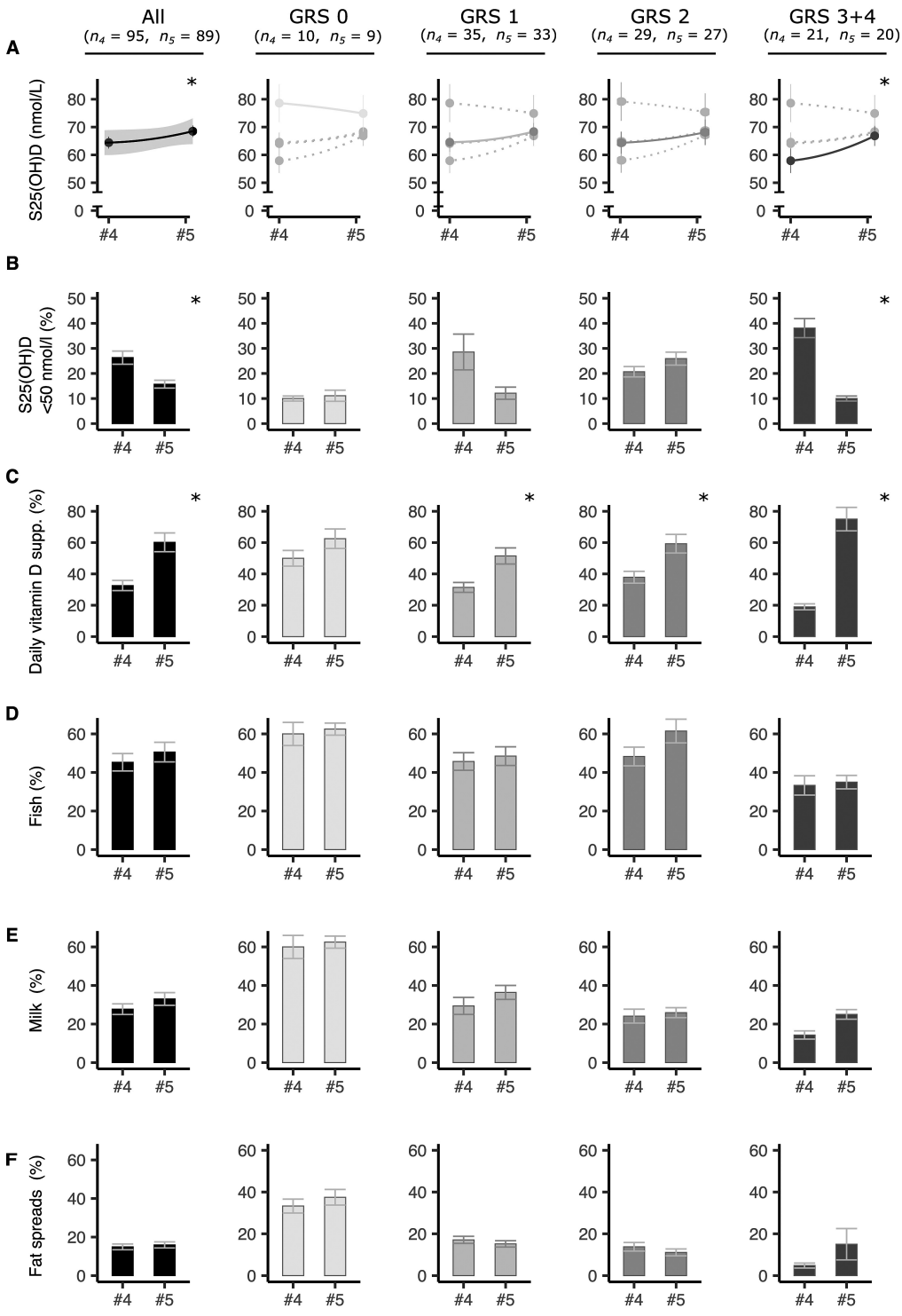

FIGURE 4 Vitamin D status of the DHR Study participants and the consumption of vitamin D supplements and vitamin D-rich foods before (study visit 4) and after (study visit 5) the personalized vitamin D supplementation and dietary guidance. Data are shown for all the participants and stratified by the GRS. (A) Mean serum 25(OH)D concentrations. The error bars represent the SEM. The respective GRS line is solid, and the lines for the other GRS groups are dotted. (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 error bars in panels B–F represent 95% CI for a sample proportion. Significant changes (P value <0.05) are marked with a star. 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. 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. There were following exceptions in the number of observations: (C) all $n_5 = 88$, GRS0 $n_5 = 8$; (D) all $n_5 = 87$, GRS0 $n_5 = 8$, GRS2 $n_5 = 26$; (E) all $n_4 = 94$, GRS1 $n_4 = 34$, all $n_5 = 88$, GRS0 $n_5 = 8$; (F) all $n_4 = 94$, GRS0 $n_4 = 9$, all $n_5 = 88$, GRS0 $n_5 = 8$. *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.

the mean serum 25(OH)D concentration increased in the group of participants with the highest GRSs (i.e., 3 or 4 risk alleles) and decreased in the participants without any risk alleles. Importantly, the association between the GRS and serum 25(OH)D concentration disappeared after the dietary and supplementation advice. This is exactly what one would expect to observe in cases where genetic characteristics can influence nutritional status.

There are significant seasonal variations in serum 25(OH)D concentrations in the Finnish population and the concentrations decrease rapidly after the summer months (68). In the DHR Study, we monitored serum 25(OH)D concentrations from August to November. Vitamin D synthesis in the skin depends on solar UVB radiation, which is diminished during the winter months (from October to March) in Northern latitudes (1). Therefore, the positive changes observed in the mean serum 25(OH)D concentrations following the guidance were not likely due to variation in sun exposure.

Our results indicate that individuals with multiple risk alleles predisposing to low serum 25(OH)D could require more 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, because the risk of harmful effects is low up to the dose of 100 μg/d (23), even higher supplementation levels than those applied in our study could be used (16).

As expected based on the guidance given on the supplement use, we observed a significant increase in daily or almost daily vitamin D supplement users, especially in participants with multiple risk alleles and lower mean serum 25(OH) concentration. There were no significant changes in the consumption of vitamin D–rich foods despite the recommendations and room for improvement. It could 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 might 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 participants with the highest risk of low vitamin D status considered the GRS information important regarding their health. Our results show that returning the GRS for serum 25(OH)D directly to individuals can be accomplished safely and this can help to tailor personalized vitamin D supplementation and improve vitamin D status. This could, in return, decrease the risk of osteoporosis and potentially other diseases.

Testing of serum 25(OH)D concentration in the general population and subsequent supplementation with vitamin D have been debated (26, 28, 72, 73). Many researchers have been concerned that large-scale testing of serum 25(OH)D and subsequent supplementation increase the burden and costs of the healthcare system (28, 73). For example, Pilz et al. (28) concluded that measuring serum 25(OH)D should not be used as a population-wide screening tool but applied only in selected individuals at high risk of vitamin D deficiency. Our proof-of-concept study provides several insights into this debate with a novel precision medicine angle. 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 to healthcare if such systems were routinely implemented. Proper consultation and personal advice should still be offered as a backup. Second, our data suggest that the knowledge of the genetic propensity for low serum 25(OH)D, combined with dietary and supplementation data, could help to identify individuals who would most likely benefit from screening serum 25(OH)D. A recent study by Hatchell et al. (74) also suggested that polygenic risk scores could be used as predictive tools for determining serum 25(OH)D concentrations and personalized vitamin D supplementation. Our results were based on the Finnish population, but the model could be modified to include more genetic data and be tailored to other populations, geographical regions, and countries. Third, as patients and healthy people are diagnosed with low vitamin D status in the healthcare setting, the genetic risk could be determined simultaneously. This would provide a systematic view of how long-term supplementation could be carried out, potentially reducing the need for repeated measurements of serum 25(OH)D concentration and repeated healthcare consultations. This could be critically important to ensure that patients with major symptoms or high risk of osteoporosis receive sufficient vitamin D supplementation. Finally, in the future, more and more people will get access to their genome data from clinical tests, population studies, or personalized genomic services. We suggest including a GRS for vitamin D status in such panels, because this measurement probably carries a favorable risk-benefit ratio and could be combined with dietary and vitamin supplementation advice.

This study was designed to test procedures as well as attitudes of people, and the results need to be validated. Our study had a longitudinal design that originated from the DHR Study, including 5 study visits during 16 mo, and focusing on a cohort that is rather homogeneous geographically, ethnically, genetically, and in terms of diet and lifestyle. We think that these advantages partly compensate for the relatively small size of the DHR Study cohort. Furthermore, the use of the much bigger, independent, and population-matched H2011 cohort was important. There were also comprehensive data available on most of the known risk factors for low serum 25(OH)D concentration from both cohorts.

In conclusion, the GRS we developed helped to identify individuals who are genetically predisposed to low serum 25(OH)D concentration and, therefore, benefit most from monitoring serum 25(OH)D as well as from a higher vitamin D intake. This study shows that direct return of data on individual genetic risk for low vitamin D status, serum 25(OH)D concentrations, and lifestyle via a web or smartphone application can be used to guide personalized vitamin D supplementation and thereby promote individualized health.

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The authors' responsibilities were as follows—RJS, MAK, HV, and OK: designed research; RJS, RDM, and TAM: conducted research; TEJ, AL, EK, HK, JIK, and CL-A: provided essential materials; RJS, OD, and SR: analyzed data and performed statistical analysis; RJS, OD, SR, MAK, and OK: wrote the paper; RJS: had primary responsibility for final content; and all authors: read and approved the final manuscript.

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