Large Individual Differences in Serum 25-Hydroxy-vitamin D Response to Vitamin D Supplementation: Effects of Genetic Factors, Body Mass Index, and Baseline Concentration. Results from a Randomized Controlled Trial

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Abstract
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The main aim of the study was to determine the influence of genetic factors on the serum 25-hydroxyvitamin D response to vitamin D supplementation. The main outcome measure was an increase in serum 25-hydroxyvitamin D after vitamin D supplementation. The patients are part of a randomized controlled trial in individuals with prediabetes assigned to 20 000 IU of vitamin D_3 per week or placebo for 12 months. A total of 484 subjects were included in the analyses and genotyped for single nucleotide polymorphisms in the DBP, DHCR7, CYP2R1, and CYP24A1 genes. Single nucleotide polymorphisms from all 4 selected genes were significantly related to baseline serum 25-hydroxyvitamin D concentrations with differences between major and minor homozygote genotypes ranging from 4.4 to 19.2 nmol/l. In the subjects given vitamin D, those with genotypes with the highest baseline 25-hydroxyvitamin D concentration also had the highest 25-hydroxyvitamin D concentration after 12 months, and the increase (delta) in 25-hydroxyvitamin D was significantly related to 3 of the single nucleotide polymorphisms. The increase in serum 25-hydroxyvitamin D was also higher in lean vs. obese subjects, and higher in those with low baseline 25-hydroxyvitamin D concentrations. When combining these 3 factors in a linear regression model, the predicted (and observed) difference in 25-hydroxyvitamin D increase between high and low responders to the supplementation was approximately 60 nmol/l. In conclusion, due to genetic, body mass, and baseline 25-hydroxyvitamin D differences, there are huge individual variations in the serum 25-hydroxyvitamin D response to vitamin D supplementation that could be of clinical importance.

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Introduction
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The nuclear vitamin D receptor (VDR) is found in cells in a number of tissues, and the enzyme necessary for the activation of 25-hydroxyvitamin D [25(OH)D] to the active form 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] is present also in extrarenal tissues [1]. Accordingly, vitamin D is likely to be important for more than skeletal health, and low serum 25(OH)D levels, used to evaluate a subject's vitamin D status, are associated with a number of adverse health outcomes [2]. Recent guidelines recommend serum levels of at least 50–75 nmol/l [3,4], and if so, billions of people are vitamin D deficient and in need of vitamin D supplementation [5].

However, there are several reports on a U- or J-shaped relation between serum 25(OH)D levels and health effects [6–8], with an optimal serum 25(OH)D level of 50–60 nmol/l in one study [8]. When giving recommendations for vitamin D supplementation to the general public, it is therefore important to know not only if there are subgroups in need of higher doses than average, but also if there are subgroups where the increase in serum 25(OH)D will be particularly high.

Individual factors affect the 25(OH)D response to vitamin D supplementation, and obese subjects need higher doses to achieve a desired increase [9, 10]. Genetic factors may also be important, and genome wide association studies (GWAS) have shown that single nucleotide polymorphisms (SNPs) in the vitamin D binding protein (DBP), as well as in enzymes necessary for activation or degradation of vitamin D and its metabolites, affect serum 25(OH)D concentration [11,12]. So far most studies on 25(OH)D response to vitamin D have been dose-response studies. Few

studies have included genetic and other factors in the analyses [13–15], and the results have not been conclusive. We are presently performing a large randomized controlled trial (RCT) with vitamin D where we have relevant genetic and background data available, as well as the one year 25(OH)D responses, and therefore had the opportunity to address these questions.

Materials and Methods
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Study design
The design of the study and the study population have been described in detail previously [16]. Briefly, the subjects are participants in an ongoing RCT with vitamin D vs. placebo in subjects with prediabetes that runs over 5 years with annual oral glucose tolerance tests. 5 hundred and eleven subjects entered the study at baseline, 256 received 20 000 IU of vitamin D_3 (Dekristol, Mibe, Jena, Germany) per week and 255 received placebo capsules that looked identical (Hasco-lek, Wroclaw, Poland). Thirty capsules were supplied at baseline and after 6 months to ensure sufficient supply if a visit had to be delayed. Unused medication was returned and counted. Compliance rate (%) was calculated as the ratio between capsules used/study weeks. In this calculation, those who had used more capsules than the intended one per week (based in the number of capsules returned) had their compliance set to 100%.

The subjects included had to be between age 21–80 years and have impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) as defined by World Health Organization [17]. Exclusion criteria were primary hyperparathyroidism, sarcoidosis or other granulomatous disorders, urolithiasis, cancer during the 5 last years, reduced kidney function, or unstable angina pectoris, acute myocardial infarction or stroke in the last year. Fertile women had to use contraception, could not be pregnant or be lactating. The subjects were not allowed to take vitamin D supplements (including cod liver oil) exceeding 400 IU/day during the study. A sum of 484 subjects came to the one-year visit and were included in the present study.

In order to ensure that all investigators remained blinded, all data were sent directly to the Hospital's Research Department where the data files were merged and coupled to the randomization code. The Research Department then sent the final file without person identification to the principal investigators (S.T.S. and R.J.).

Fasting serum samples were drawn at baseline and after 12 months. Previously the measurements of serum levels of 25(OH)D, serum calcium, parathyroid hormone (PTH), and HbA1c have been described [16,18]. Height and weight were measured wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m²). Questionnaires on intake of vitamin D supplements including cod liver oil and calcium supplements were filled in at baseline and after 12 months.

Selection of SNPs for analysis
The serum 25(OH)D concentrations are related to SNPs in the DBP gene (DBP or GC) responsible for binding and transportation of vitamin D metabolites in the circulation, in the 7-dehydrocholesterol (7-DHC) reductase gene (DHCR7) responsible for the availability of vitamin D precursor 7-DHC in the skin, in the 25-hydroxylase gene (CYP2R1) involved in the conversion of vitamin D into 25(OH)D in the liver, and in the 24-hydroxylase gene (CYP24A1) involved in the degradation of 25(OH)D [19]. To avoid problems with multiple testing, we selected one SNP in each of these genes and chose the one with the highest difference in serum 25(OH)D between the major and minor homozygote genotype (rs2282679, rs3829251, rs10741657, rs6013897, respectively) as reported in our previous studies [14]. In addition, we included two SNPs in the DBP gene (rs7041 and rs4588) since reference to these two SNPs are frequently made in relation to serum 25(OH)D levels [11,12,20]. Blood samples for SNP analyses were sent to KBiosciences (http://www.lgcgenomics.com/genotyping/) and all genotyping were performed with a competitive allele-specific polymerase chain reaction (KASPar) assay that enables highly accurate bi-allelic scoring of SNPs.

Statistical analyses
Normal distribution was evaluated by means of Kolmogorov–Smirnov test and Q-Q plots. Serum PTH was not normally distributed and was log-transformed when used in the statistical analyses. Data are presented as mean±SD for normally distributed variables and as median (2.5th, 97.5th percentiles) for non-normally distributed variables. Trends across the genotypes were evaluated using linear regression with age, sex, and BMI as covariates. For the baseline values, season (summer (May–September)/winter (October–April)) and intake of vitamin D supplements (including cod liver oil), were also included as covariates.

The genotype frequencies were examined for compliance with Hardy-Weinberg equilibrium using χ^2 analysis [21]. The linkage disequilibrium (LD) between SNPs was evaluated with r^2 using CubeX calculations with $r^2 \geq 0.4$ as a cutoff for LD [22]. Level of significance was set at $p < 0.05$ (two-tailed). All statistical analyses were performed using IBM SPSS Statistics version 21.

Ethics
The study was approved by the Norwegian Medicines Agency and by the Regional Committee for Medical Research Ethics. The trial including the genetic analyses was registered at ClinicalTrials.gov (NCT00685594). However, the analysis of serum 25(OH)D response to supplementation in relation to genetic polymorphisms was not explicitly pre-specified.

Results
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The two DBP SNPs rs2282679 and rs4588 were in LD with each other ($r^2 = 0.98$), and rs4588 was therefore not included in further analyses. None of the other SNPs were in LD, and all SNPs were in Hardy-Weinberg equilibrium, χ^2 testing; $p > 0.05$. There were no reports or observations of serious adverse events during the one-year study period.

Baseline 25(OH)D levels
The baseline characteristics of the 484 subjects are presented in ○ **Table 1**, the vitamin D and placebo groups did not differ significantly. As expected the serum 25(OH)D levels were higher during the summer than the winter months, 64.8 nmol/l±22.0 vs. 57.3 nmol/l±20.6, $p < 0.001$, and females had higher 25(OH)D concentrations than males, 64.5 nmol/l±22.6 vs. 58.4 nmol/l±20.6, $p < 0.01$. The serum 25(OH)D concentrations were only slightly, and nonsignificantly, higher in those using vitamin D supplementation vs. those not using supplements, 62.0 nmol/l±19.3 vs. 60.0 nmol/l±22.5. The distributions in baseline concentration are shown in ○ **Fig. 1**.

Table 1   Baseline characteristics in all subjects and in relation to randomization status.

Variables	All	Randomization status	
		Vitamin D	Placebo
Male/Female	298/186	152/90	146/96
Age (years)	62.0 ± 8.6	62.1 ± 8.2	62.0 ± 9.1
BMI (kg/m^2)	29.9 ± 4.3	30.1 ± 4.1	29.8 ± 4.4
HbA1c (mmol/mol) *	41.8 ± 3.4	41.9 ± 3.3	41.7 ± 3.5
Serum 25(OH)D (nmol/l)†	60.7 ± 21.6	59.8 ± 21.9	61.6 ± 21.2
Serum calcium (mmol/l)‡	2.31 ± 0.08	2.31 ± 0.07	2.31 ± 0.08
Serum PTH (pmol/l)§	5.68 ± 2.23	5.78 ± 2.29	5.58 ± 2.17
Daily vitamin D supplementation (no of subjects/mean dose in IU for subjects taking supplements) *	163/335 ± 153	77/310 ± 127	86/358 ± 170

 * Data missing for 8 subjects (6 in the vitamin D group and 2 in the placebo group)
† Data missing for 2 subjects (1 in both the vitamin D group and the placebo group)
‡ Data missing for 1 subject in the placebo group
§ Data missing for 5 subjects (2 in the vitamin D group and 3 in the placebo group)
BMI: Body mass index; HbA1c: Glycated hemoglobin; 25(OH)D: 25-Hydroxyvitamin D; PTH: Parathyroid hormone
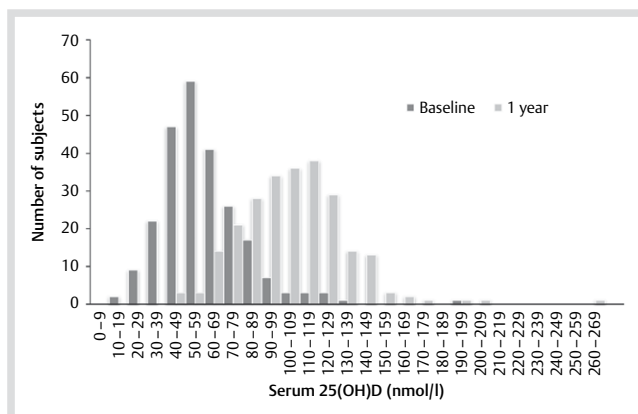

Fig. 1   The distribution in baseline and in 12 month serum 25(OH)D concentration in subjects randomized to vitamin D.

For all five SNPs there was a significant effect of genotype on the serum 25(OH)D concentration with the difference between major and minor homozygote genotypes ranging from 4.4 to 19.2 nmol/l (**Supplemental Table S1**). None of the SNPs were related to sex, age, BMI, or serum calcium (**Supplemental Table S1**). However, there was a significant relation between rs6013897 and PTH, with the highest serum PTH in the subjects with the minor homozygote genotype who also had the lowest serum 25(OH)D (**Supplemental Table S1**). This significant relation between rs6013897 and PTH was also seen in the subjects given vitamin D after 1 year (data not shown).

Serum 25(OH)D levels after one year
The mean compliance rate in both the vitamin D and the placebo groups was 97.5 %. Subjects in the vitamin D group had a significant increase in mean serum 25(OH)D from baseline levels of 59.8 nmol/l ± 21.9 to 105.6 nmol/l ± 27.7 after 12 months (**Table 2**); however, still 12.4 % had serum 25(OH)D levels < 75 nmol/l and a large spread in 12 month and delta 25(OH)D concentrations were seen (**Fig. 1,2**). In the placebo group, the serum 25(OH)D concentrations did not change significantly (**Supplemental Table S2**).

Effect of genotype on serum 25(OH)D concentrations after one year
The increase in serum 25(OH)D in the vitamin D group was highly dependent on genotype. For all the five SNPs the genotype with

the highest baseline 25(OH)D concentration also had the highest 25(OH)D concentration after 12 months, and for 4 of the SNPs the difference in 25(OH)D between the major and minor homozygote genotypes increased (range 15.9–28.2 nmol/l) after vitamin D supplementations (**Table 2**). For 3 of the SNPs (rs2282679, rs7041, and rs10741657) there was a significant relation between genotype and increase (delta) in 25(OH)D with differences between major and minor homozygote in delta 25(OH)D being 6.3, 11.9 and 13.8 nmol/l, respectively (**Table 2**).

Effect of sex, age, BMI, and baseline 25(OH)D on serum 25(OH)D after vitamin D supplementation
At baseline females had significantly higher 25(OH)D concentration than males, but they had an almost identical increase in serum 25(OH)D after 12 months (**Table 2**). There was a clear relation between age and serum 25(OH)D at baseline with the highest concentration in the oldest subjects. The same was seen if excluding subjects taking vitamin D supplements (data not shown). However, the increase in serum 25(OH)D appeared unrelated to age (**Table 2**).

There was a significant and inverse relation between BMI and increase in 25(OH)D concentration after supplementation. In spite of 6.9 nmol/l higher baseline 25(OH)D levels, subjects with BMI < 25 kg/m^2 had a 18.6 nmol/l higher increase in 25(OH)D than subjects with BMI > 35 kg/m^2 (**Table 2**).

Subjects with the lowest baseline concentration of 25(OH)D had the highest increase in serum 25(OH)D (**Table 2**). Thus, subjects with baseline serum 25(OH)D concentration < 40 nmol/l had a 24.1 nmol/l higher increase in 25(OH)D than those with baseline serum 25(OH)D > 75 nmol/l. However, after 12 months supplementation with vitamin D their mean serum 25(OH)D levels were still 36.6 nmol/l lower compared to those with baseline serum 25(OH)D > 75 nmol/l (**Table 2**).

Accordingly, in a multiple linear regression model with age, BMI, sex, and baseline serum 25(OH)D levels as covariates, only BMI and baseline 25(OH)D concentration were significant and negative predictors of increase in serum 25(OH)D. There was no effect of calcium supplementation on the serum 25(OH)D increase (data not shown).

Relation between baseline and 1 year serum 25(OH)D levels in those given placebo
There was a high correlation between baseline and 12 months serum 25(OH)D concentrations in those given placebo (r = 0.70, p < 0.001) (data not shown), but a clear regression towards the

Variables	n	Baseline serum 25(OH)D (nmol/l)	12-Month serum 25(OH)D (nmol/l)	Delta serum 25(OH)D (nmol/l)
SNP genotypes				
rs2282679				
Major homozygote	150	63.0 ± 23.1 *	112.7 ± 28.8 *	50.0 ± 25.4 *
Heterozygote	77	57.4 ± 19.0	95.9 ± 21.9	38.5 ± 21.0
Minor homozygote	15	40.8 ± 10.6	84.5 ± 15.8	43.7 ± 16.6
rs7041‡				
Major homozygote	77	62.6 ± 25.4 *	117.0 ± 30.9 *	54.4 ± 26.5 *
Heterozygote	117	61.4 ± 20.5	103.0 ± 25.3	41.8 ± 22.8
Minor homozygote	47	50.8 ± 16.4	93.2 ± 20.9	42.5 ± 20.0
rs3829251§				
Major homozygote	134	63.2 ± 23.5 *	108.2 ± 28.9†	45.0 ± 23.8
Heterozygote	84	55.4 ± 19.5	101.6 ± 25.4	46.5 ± 23.6
Minor homozygote	18	55.4 ± 18.2	102.7 ± 23.3	47.3 ± 24.6
rs10741657				
Major homozygote	77	57.4 ± 25.5	100.1 ± 30.4 *	42.7 ± 23.2 *
Heterozygote	119	60.6 ± 18.9	104.2 ± 22.7	43.8 ± 20.9
Minor homozygote	47	61.7 ± 22.7	118.2 ± 31.1	56.5 ± 30.1
rs6013897‖				
Major homozygote	138	62.6 ± 23.0 *	108.1 ± 30.3 *	45.8 ± 24.9
Heterozygote	88	57.2 ± 20.9	103.7 ± 24.1	46.4 ± 23.4
Minor homozygote	14	50.2 ± 11.9	92.2 ± 18.8	42.0 ± 22.0
Sex				
Male	152	58.2 ± 22.8†	104.0 ± 28.6	45.8 ± 25.9
Female	90	62.6 ± 20.2	108.4 ± 26.1	46.2 ± 25.9
Age group				
<50 years	22	51.5 ± 18.9 *	95.3 ± 27.8	43.8 ± 27.7
50–59 years	50	56.0 ± 21.4	106.9 ± 26.8	50.9 ± 26.0
60–69 years	133	60.4 ± 21.9	106.3 ± 28.5	46.1 ± 23.0
>70 years	37	67.9 ± 22.1	107.8 ± 25.6	39.8 ± 22.5
BMI group				
<25 kg/m²	23	59.0 ± 14.9	119.3 ± 34.3 *	60.4 ± 32.9†
25–29.9 kg/m²	110	59.7 ± 21.5	106.9 ± 25.1	47.5 ± 24.2
30–34.9 kg/m²	78	63.2 ± 24.5	104.4 ± 29.4	41.2 ± 21.7
>35 kg/m²	31	52.1 ± 19.6	93.9 ± 22.5	41.8 ± 17.3
Baseline 25(OH)D				
<40 nmol/l	33	32.0 ± 6.3	90.5 ± 26.8	58.5 ± 26.5 *
40–49 nmol/l	47	44.9 ± 3.2	91.9 ± 18.0	47.0 ± 17.8
50–74 nmol/l	114	60.4 ± 6.8	107.1 ± 23.9	46.6 ± 24.0
>75 nmol/l	47	92.7 ± 21.6	127.1 ± 30.9	34.4 ± 23.6

Table 2 Serum 25(OH)D at baseline, 12-month, and delta values in relation to vitamin D SNP genotypes, sex, age, BMI, and baseline 25(OH)D categories in subjects randomized to vitamin D supplementation.

* p<0.01 and † p<0.05

Baseline: Linear trend across the genotypes with sex, age, BMI, season, and intake of vitamin D supplementation as covariates. Twelve month and delta values: Linear trend across the genotypes with sex, age, and BMI as covariates. Normally distributed data are presented as means ± SD

‡ Data missing for 1 subject

§ Data missing for 6 subjects

‖ Data missing for 2 subjects

SNP: Single nucleotide polymorphism; BMI: Body mass index; 25(OH)D: 25-Hydroxyvitamin

mean with an increase of 8.6 nmol/l in serum 25(OH)D in those with baseline serum 25(OH)D<40 nmol/l and a decrease in serum 25(OH)D of 7.9 nmol/l in those with baseline serum 25(OH)D>75 nmol/l (**Supplemental Table S2**).

Prediction of change in serum 25(OH)D concentration based on baseline 25(OH)D concentration, baseline BMI, and genotype

To predict change in serum 25(OH)D concentration based on baseline 25(OH)D concentration, baseline BMI, and genotype, we set up a regression equation with randomization status, baseline BMI and baseline serum 25(OH)D and the three SNPs that in the model were significant (rs2282679, rs7041, and rs10741657) and interaction terms between each of the three SNPs with the randomization status: delta 25(OH)D = intercept + (β-randomization status × randomization status) + [β-baseline 25(OH)D × baseline 25(OH)D] + (β-baseline BMI × baseline BMI) + (β-rs2282679 × rs2282679) + (β-rs10741657 × rs10741657) + (β-rs7041 × rs7041) + [randomization status × baseline BMI × (β-randomization status × rs2282679)] + [randomization status × baseline BMI × (β-randomization status × rs10741657)] + [randomization status × baseline BMI × (β-randomization status × rs7041)]. Sex and age did not significantly influence delta 25(OH)D and were therefore not included in the equation.

The resulting delta 25(OH)D responses according to baseline vitamin D status and BMI and "best" [associated with highest increase in serum 25(OH)D] and "worst" [associated with the lowest increase in serum 25(OH)D] genotypes are shown in ◉ **Table 3**. As an example of extreme difference in response, a lean person with BMI 22 kg/m², baseline 25(OH)D concentration

40 nmol/l and all 3 "best" genotypes (major homozygote for rs2282679 and rs7041 and minor homozygote for rs10741657) was estimated to have a delta 25(OH)D of 77.8 nmol/l, while a person with the same baseline 25(OH)D but BMI 40 kg/m² and with the "worst" genotypes (minor homozygote for rs2282679 and rs7041 and major homozygote for rs10741657) was estimated to have a delta 25(OH)D of about 19.3 nmol/l.

Discussion
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In the present intervention study, we have found that the baseline serum 25(OH)D concentration is influenced by genetic factors, and that these factors together with BMI and the baseline 25(OH)D concentration are strong predictors of the serum 25(OH)D response to vitamin D supplementation. Humans get vitamin D from the diet (fatty fish), cod liver oil, vitamin D supplements, and from endogenous production in the skin upon solar UVB exposure [2]. The serum 25(OH)D concentration is mainly the result of available vitamin D as substrate for 25-hydroxylation in the liver, binding and transportation in the circulation by DBP, and degradation by 24-hydroxylation and subsequent removal from the circulation [2]. It is therefore no surprise that SNPs in genes related to synthesis (*DHCR7/NADSYN1* and *CYP2R1*), binding and transportation (*DBP/GC*), and degradation (*CYP24A1*) affect the 25(OH)D concentration, which has been shown in several GWAS studies [11, 12]. The differences we have found between major and minor homozygote's for these SNPs are very similar to those reported

Fig. 2   The distribution in increase (12 month minus baseline value) in serum 25(OH)D concentration in subjects randomized to vitamin D.

by others [11, 12, 14, 20, 23–26]. We have also previously published similar results based on 3 smaller RCTs where only 3 of the present SNPs (rs2282679, rs7041, rs10741657) were significantly associated with serum 25(OH)D [14].

The most remarkable genotype effect was for the SNPs in the *DBP/GC* gene. Thus, for rs228279 the subjects with the major homozygote genotype had 19.2 nmol/l, or 43.6%, higher serum 25(OH)D concentration than those with the minor homozygote genotype. In spite of this difference, there was no significant relation with serum PTH, which is a good marker of vitamin D's biological effects [27]. However, SNPs in the *DBP/GC* gene are related not only to the total serum 25(OH)D concentration, but also to the serum level of DBP and/or DBP phenotype [28, 29]. DBP exist in 6 major phenotypes, each with different binding coefficients for 25(OH)D [30, 31], and accordingly, the total 25(OH)D may not accurately reflect the level of free or bioavailable (sum of free and albumin-bound) 25(OH)D [32]. If these 2 latter 25(OH)D fractions are the ones responsible for biological activity, then that could explain the lack of association between rs228279 and rs7041 and PTH. On the other hand, for the *CYP24A1* SNP there was a highly significant association with serum PTH, both when analyzing all subjects at baseline as well as in the vitamin D group after 12 months. This is unlikely to be a chance finding since we have reported a similar result in a cohort of 9 471 subjects [23].

Accordingly, the biological importance of genotype-associated differences in total serum 25(OH)D is uncertain and may depend on the SNP in question. This will probably first be settled when it is clarified which vitamin D metabolite [total 25(OH)D, free and/or bioavailable 25(OH)D, or even the mother compound vitamin D], one should measure to evaluate a subject's vitamin D status. Until then, measurement of total serum 25(OH)D concentration will remain the gold standard regardless of what recommendations concerning sufficient and/or optimal serum concentrations are needed for supplementation.

It is generally assumed that an intake of 100 IU/day leads to an increase in serum 25(OH)D of approximately 2.5 nmol/l [33]. However, the response to supplementation varies considerably from person to person resulting in a very wide distribution for serum 25(OH)D concentrations after 1 year as illustrated in ○ **Fig. 1**. The most important predictor for the response to supplementation was in our study genetic factors. Thus, for all five SNPs the increase in serum 25(OH)D after supplementation was greatest in the genotypes with the highest 25(OH)D at baseline. This is no surprise, since genetic differences in production, transportation and degradation of 25(OH)D resulting in differ-
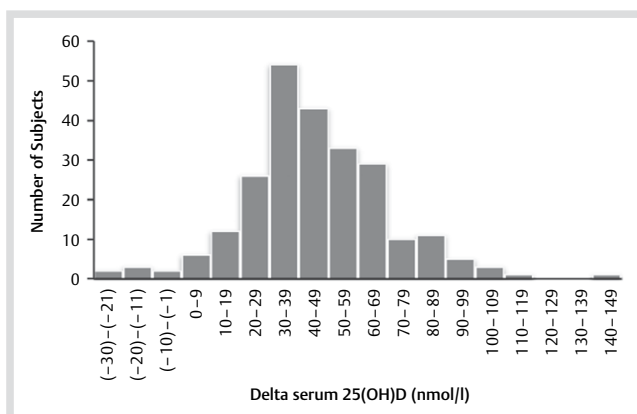
Table 3   Predicted increase (delta) in 25(OH)D (nmol/l) according to baseline 25(OH)D, BMI, and genetic status after supplementation with 20 000 IU per week for one year.

Variables	BMI 22 kg/m²		BMI 25 kg/m²		BMI 30 kg/m²		BMI 35 kg/m²		BMI 40 kg/m²	
	Best genes	Worst genes	Best genes	Worst genes	Best genes	Worst genes	Best genes	Worst genes	Best genes	Worst genes
Baseline serum 25(OH)D										
30 nmol/l	81.2	47.5	77.0	43.3	70.1	36.4	63.2	29.5	62.3	22.6
40 nmol/l	77.8	44.1	73.7	40.0	66.8	33.1	59.9	26.2	53.0	19.3
50 nmol/l	74.5	40.8	70.3	36.6	63.4	29.7	56.5	22.8	49.6	15.9
60 nmol/l	71.1	37.4	67.0	33.3	60.1	26.4	53.2	19.5	46.3	12.6
70 nmol/l	67.8	34.1	63.6	29.9	56.7	23.0	49.8	16.1	42.9	9.2

"Best genes": The genes associated with the largest increase in serum 25(OH)D concentration when giving vitamin D supplementation
"Worst genes": The genes associated with the lowest increase in serum 25(OH)D concentration when giving vitamin D supplementation
25(OH)D: 25-Hydroxyvitamin D; BMI: Body mass index

ences in 25(OH)D at baseline will also affect the handling of the additional vitamin D supplements. Thus, subjects with genotypes associated with low serum 25(OH)D concentrations will need a higher supplemental dose to reach a higher targeted 25(OH)D level. The exception to this was the *DHCR71* SNP rs3829251, where the increase in 25(OH)D was highest in those with the genotype with the lowest baseline levels. The reason might be that this SNP is involved in conversion of 7-DHC in the skin to precursors for vitamin D production [19], and therefore probably not involved in the metabolism of exogenous vitamin D. There are few previous reports on genetic effects on 25(OH)D response to vitamin D supplementation. In 2 recently published studies, a number of SNPs were tested for relation to 25(OH)D levels [34, 35]. The most strongly related were the ones in the *CYP2R1* gene, and in the largest study by Barry et al. where 1 787 non-Hispanic whites were included, a SNP in the *VDR* gene was also found to affect the increase in serum 25(OH)D [35]. Furthermore, in their regression model for prediction of 25(OH)D response, Waterhouse et al. found inclusion of SNPs to be as important as personal and environmental factors [34].

In addition to genetic factors, the baseline concentration and BMI were also important for the 25(OH)D response. Thus, those with low baseline levels had the highest increase in 25(OH)D after supplementation, which could partly be ascribed to the expected regression towards the mean. It is also reasonable to assume that available vitamin D is metabolized slower the lower the 25(OH)D concentrations are, but in spite of these factors, those with low baseline 25(OH)D did not catch up with those who started out higher.

The serum 25(OH)D concentrations are lower in obese subjects, which could be due to lower intake of vitamin D, less sun-exposure, degradation of vitamin D in adipose tissue, or simply that obese subjects have a higher distribution volume for vitamin D [36]. Obese subjects also had a markedly reduced response to vitamin D supplementation in our study similar to that reported by others [9, 10, 37, 38], which supports storage and/or degradation of vitamin D in adipose tissue.

On the other hand, we found age, sex and calcium intake not to be important in this regard. For age, most studies report lower levels in older subjects [39, 40]; however, this is not seen in studies from our area, Northern Norway [41]. This is most probably due to a more traditional and vitamin D healthy diet with a high intake of fatty fish. In other populations where the 25(OH)D level is more related to sun-exposure, the effect of age will be different since it is established that the capacity for vitamin D production in the skin is reduced with age [39]. However, our data where the increase in 25(OH)D was similar in the age groups studied, as also found by Gallagher et al. [42], may indicate that aging does not affect the absorption and metabolism of vitamin D from diet and supplements. We saw a slight effect of sex on baseline 25(OH)D concentration, but similar response to vitamin D supplementation, and accordingly males and females probably need similar amounts of vitamin D supplementation, which was also the conclusion in the study by Aloia et al. [43]. There are reports that the intake of calcium has a vitamin D sparing effect by reducing the serum PTH concentration resulting in less hydroxylation of 25(OH)D to the active form $1,25(OH)_2D$ [44, 45]. This was not seen in our study, but the number of subjects taking calcium supplements was low.

The 3 important factors for the vitamin D supplementation response; baseline 25(OH)D concentrations, BMI, and genotype, are at least partly interrelated. We therefore integrated these factors (as well as the randomization status to account for the regression towards the mean) in a regression equation to predict the 25(OH)D response to a weekly dose of 20 000 IU vitamin D_3. When using this equation the predicted differences between sub-groups were remarkable as illustrated in ○ **Table 3**. Thus, if aiming at a serum level of >75 nmol/l by giving 20 000 IU per week, all subjects with BMI of 22 kg/m^2 or "optimal" genetic status would reach the target regardless of other factors. On the other hand, hardly any of the subjects with morbid obesity (BMI ≥ 35 kg/m^2), the "worst" genes, and a baseline serum 25(OH)D of <50 nmol/l would reach the target. In this context, it should be remembered that the frequency of the "best" alleles are higher than for those associated with low serum 25(OH)D. However, at the population level subjects with the "worst" alleles still amount to millions of subjects, and in our study 12.4 % had 1 year serum 25(OH)D levels <75 nmol/l. Also, some subjects had a remarkably high 25(OH)D response and 9.1 % had serum 25(OH)D >140 nmol/l after 1 year, which may not be favorable [6–8].

The 25(OH)D response to supplementation can be predicted by baseline concentration and BMI, which is inexpensive, but also needs genotyping, which is costly. Therefore, for the individual subject the most easiest way to tailor the vitamin D dose would be to simply measure the responding 25(OH)D concentration. However, for general advice on supplementation it is important to know that some subgroups need substantially more vitamin D to reach the desired 25(OH)D target. In this context, it should also be mentioned that at present we do not know what is an adequate 25(OH)D concentration, and recommendations differ with 50 %; the Institute of Medicine finding no proof for additional benefit with levels higher than 50 nmol/l [4], whereas guidelines from the Endocrine Society recommend a level of 75 nmol/l [3]. Furthermore, it is not known how large the therapeutic window for vitamin D supplementation is, and the U- or J-shaped relation between serum 25(OH)D and health outcomes with possible harmful effects with the higher 25(OH)D concentrations are based on association studies only.

Our study has some limitations. The study population is homogenous, but the results cannot be generalized as all participants had IGT and/or IFG, live in Northern Norway at latitude of 69 ° with low UVB solar exposure, and almost all were Caucasians. Furthermore, we did not include data on sun exposure, skin darkness, and physical activity, and we did not measure the free fraction of serum 25(OH)D, which might be the biologically active one. Nor did we measure the 25(OH)D catabolite 24,25-dihydroxyvitamin D [24,25(OH)$_2$D] which could have been of importance since it has been reported that the 24,25(OH)$_2$D/25(OH)D ratio predicts the 25(OH)D response to vitamin D supplementation [46].

However, the study also has strength, as we included a large group of subjects, had predefined a limited number of SNPs to evaluate, and were able to create an applicable regression equation for predicting response to vitamin D supplementation.

In conclusion, we do know that lifestyle is the most important determinant for 25(OH)D concentration. However, it is difficult to change an unhealthy lifestyle, and if the present recommendations regarding adequate 25(OH)D concentrations are correct, a considerable number of subjects need vitamin D supplementation. There are large individual differences in response to supplementation, and this needs to be taken into account when giving general advice on vitamin D supplementation.

Authors' Contribution
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Acknowledgements
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Conflict of Interest
▼

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

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