Impact of genetic influence on serum total- and free 25-hydroxyvitamin-D in humans.

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ABSTRACT

Serum 25-hydroxyvitamin D /25OHD/ levels in humans are determined primarily by environmental factors such as UV-B radiation and diet, including vitamin D intake. Although some genetic determinants of 25OHD levels have been shown, the magnitude of this association has not yet been clarified. The present study evaluates the genetic contribution to total- /t-25OHD/ and free-25OHD /f-25OHD/ in a representative sample of the Hungarian population (n=462). The study was performed at the end of winter to minimize the effect of sunlight, which is a major determinant of serum vitamin D levels. Single nucleotide polymorphisms (SNPs) of five genes playing major roles in vitamin D metabolism were investigated (*NADSYN1, DHCR7, GC, CYP2R1 and CYP24A1*). The selected SNPs account for 13.1% of the variance of t-25OHD levels. More than half of the genetic effect on t-25OHD levels was explained by two polymorphisms (rs7935125 in *NADSYN1* and rs2762941 in *CYP24A1*), which had not previously been investigated with respect to vitamin D metabolism. No SNPs exhibited association with f-25OHD levels. Unexpectedly, SNPs that showed univariate associations with vitamin D binding protein (DBP) levels were not associated with f-25OHD levels questioning the biological significance of these polymorphisms.

The present study shows that t-25OHD levels are significantly influenced by genetic factors, however, the clinical significance of this observation remains to be defined, as variation in f-25OHD levels are marginally explained by genetic effects.

Key Words: Epidemiology/general population studies; Genetic research/human association studies; Diseases and disorders of/related to bone/other-vitamin D deficiency.

1. Introduction

Serum 25-hydroxyvitamin D (25OHD) levels in humans are known to be determined by demographical characteristics, environmental factors, health status and lifestyle choices, including exposure to sun and artificial UV-B radiation [1], vitamin D supplementation [2], age [3], sex [2], diet [4], and body mass index (BMI) [2]. Additionally, there is some evidence that genetic makeup also influences vitamin D levels [5]. Genome-wide association (GWA) studies reveal only a minor role for genetic influence on systemic vitamin D levels – in the range of 1-4% [6, 7]. Nevertheless, previous twin and linkage studies demonstrated a 28-80% hereditability for 25OHD levels [8-11]. This apparent contradiction may be at least partly explained by the fact that environmental factors were not considered in GWA analyses, and the examined populations were genetically very heterogeneous. To the best of our knowledge, no data has been published on the association between genetic factors and 25OHD levels in a population with minimal confounding effect of environmental factors.

In clinical practice we define vitamin D adequacy based on serum total 25OHD (t-25OHD) levels. However, the majority of vitamin D circulates in a protein-bound form and – according to the free-hormone hypothesis – only the unbound (or free) form is biologically available and responsible for most of the biological effects. It is thus hypothesized that free 25-hydroxyvitamin D (f-25OHD) would better predict vitamin D deficiency than t-25OHD. As albumin only weakly bounds 25OHD, we also distinguish a bioavailable fraction: the albumin bounded and the free fractions together.

It is unclear whether genetic effects are similar in size on t-25OHD, f-25OHD or on biologically available (b-25OHD) levels, or the same or different SNPs are responsible for these effects.

The present survey done on a representative sample of the Hungarian population was designed to minimize the environmental effects on serum t-25OHD levels, therefore improving the chance of revealing any genetic determinants. This purpose was reached by conducting sample

collection at the end of the five-month long winter. The fact that most Hungarian foods are low in vitamin D [12] and not fortified with this compound gives us confidence that dietary effects are also controlled for. Furthermore, subjects chronically exposed to other significant environmental factors were excluded. Thus, this investigation was conducted to achieve three major goals, briefly summarized as follows: first, to characterize genetic influences on serum t-25OHD, f-25OHD, and b-25OHD variability in a representative general population with minimal interference from non-genetic confounders; second, to analyze the association between the SNPs of genes playing role in vitamin D metabolism and serum t-25OHD levels; and third, to evaluate the association between the above SNPs in vitamin D metabolic pathway-associated genes and f-25OHD and b-25OHD levels.

2. Materials and methods

2.1. Subjects and Setting

Participants were drawn from a representative cross-sectional survey of the Hungarian adult (≥18 years of age) community dwelling population [13]. We used a two-stage sampling technique to draw our subjects. First, using the 2012 general practitioner list of the National Public Health Officer Service, we invited a stratified random sample of 20 GP practices of whom 15 provided data for our analyses. The second stage involved a non-probability sampling of people enlisted in the GP registry using a stratified quota sampling that corresponded to the age and sex structure of the Hungarian adult population [13]. Blood samples were collected from all participants within a week at the end of winter to ensure that sunlight exposures both minimized and could be treated as a constant. The collection period was scheduled prior to the first consecutive sunny days of spring in Hungary. For the year 2013, this was the first week of April. The effect of natural UV-B radiation on 25OHD levels were considered minimal, since Hungary is located on the Northern hemisphere over 40˚ latitude. Thus, from late autumn until

spring, ambient solar radiation in the country has a relatively minor UV-B component, and is at a level that does not significantly contribute to physiologically relevant vitamin D synthesis [13]. The study population was ethnically homogenous. All participants in the current survey were of Caucasian origin and Hungarian citizens. Background data collected for this study showed that, at the end of winter, systemic serum t-25OHD is affected by tanning bed use, traveling abroad within 3 months, and vitamin D supplement intake. This outcome is nevertheless unaffected by the amount of time spent outdoors, age, sex, or living circumstances. Accordingly, persons who had taken vitamin D supplements, traveled abroad within 3 months, used a tanning bed or had missing data were excluded from the study.

All study participants provided written informed consent before any study-related activities were initiated. This trial was approved by the Scientific and Research Ethics Committee of the Medical Research Council of Hungary, and was carried out in compliance with the World Medical Association Declaration of Helsinki's Ethical Principles for Medical Research Involving Human Subjects.

2.2. Study related procedures

Upon entry to the study, medical histories on previous and concurrent diseases were taken and vital signs and anthropometric measurements were recorded. Lifestyle characteristics and medications that potentially effect vitamin D metabolism were assessed via a self-completed questionnaire.

Blood sampling was performed according to standardized protocols under fasting conditions. Samples were processed and biomarkers were measured on the same day. The t-25OHD was determined by a fully automatized, direct competitive chemiluminescence immunoassay method (LIAISON analyzer, DiaSorin S.p.A., Saluggia, Italy). Intra- and inter-assay coefficients of variation (CV) were 4.1%-7.7% and 7.7%-10.9%, respectively, at decreasing concentrations

(60.0-19.8 nmol/l). Functional sensitivity (5.4 nmol/l) was defined at 20%CV. The measurements of 25OHD were performed in the laboratory which participates in Vitamin D External Quality Assessment Scheme (DEQAS) and the laboratory holds a DEQAS certificate. A polyclonal rabbit anti-human Gc-globulin antibody (A0021; Dako, Agilent Technologies, Santa Clara CA; USA) was used for vitamin D binding protein (DBP) measurements with immunoturbidimetry using a fully automatized analyzer (Modular, Roche, Mannheim, Germany). This antibody reacts with all subtypes of Gc-globulin. The measuring range for this method is 25-495 mg/l, and the detection limit was 7.6 mg/l. The total CVs were 3.5-6.1% at the 405-95 mg/l range. The b-25OHD was calculated according to Powe's method [14]. For f-25OHD calculations, Bikle's formula [15] was used.

2.3. Genotyping

The SNPs in genes of proteins participating in vitamin D metabolism were selected for analysis and thus the following proteins were considered: nicotinamide adenine dinucleotide synthetase (*NADSYN1*), 7-dehydrocholesterol reductase (*DHCR7*), vitamin D binding protein (*GC*), vitamin D 25-hydroxylase (*CYP2R1*), and vitamin D 24-hydroxylase (*CYP24A1*). Since the effect of UV-B radiation was excluded by design, SNPs related to skin pigmentation were not analyzed.

Based on the level of evidence regarding the association of SNPs with t-25(OH)D levels targeted SNPs were selected in two steps. First, SNPs were selected that were found in previous studies to effect t-25OHD levels (rs4588, rs7041 of *GC*; rs4809959, rs927650, rs2209314, and rs2762939 for *CYP24A1*) [5] Second, genes from the publicly available HapMap database were selected based on their minor allele frequency (MAF) (at least 20%). This resulted in identification of the following SNPs: rs1993116, rs10500804, rs11023374 for *CYP2R1*; rs17467825, rs222054 for *GC*; rs7935125 for *NADSYN1/DHCR7* and rs4809960, rs6022999,

rs2181874, rs2585428, rs3787555, rs2244719, rs2762941 for *CYP24A1*. Among the above SNPs, rs7935125 (*NADSYN1/DHCR7*), rs222054 (*GC*), rs111023374 (*CYP2R1*), rs3787555, rs2244719, and rs2762941 (*CYP24A1*) had no published data regarding their association with vitamin D metabolism in PubMed.

DNA was extracted from EDTA-anticoagulated peripheral blood using the High Pure PCR Template Purification kit (Roche Diagnostics, GmbH, Mannheim, Germany). Genotyping was performed on a Sequenom MASSarray Analyzer 4 (Sequenom, San Diego, California, U.S.A.).

2.4. Statistical analysis

Before performing any genotyping we run a power analysis based on (1) the observed distribution of f-25OHD in the present population [13] and the published literature on a vitamin D depleted population [16] with standard deviations (SD) of 8 to 10 ng/ml and (2) differences in f-25OHD found between some of the genotypes investigated by us (2.5 to 4 ng/ml) [16]. Given an SD of 10 ng/ml and an alpha level of 0.05 we had 80% power to detect a difference in f-25OHD levels from 2.7 to 6 ng/ml at different frequencies of the minor allele (50 to 5%, respectively).

Descriptive data are given as means ± SD for continuous data, and frequencies (percentages) for categorical variables. In addition to formal tests for normality, visual inspection of histograms was performed for all continuous variables.

Altogether, we investigated 19 SNPs that were considered potentially related to vitamin D levels. We performed haplotype block analysis to investigate linkage disequilibrium, and Chi-square tests to check for presence of Hardy-Weinberg equilibrium.

Of these 19 SNPs 7 were previously reported to be associated with vitamin D levels according to GWAS studies [5], the remaining 12 SNPs were suggested to be associated with vitamin D metabolism but had no known association with vitamin D levels. P-values were corrected for

multiple testing using the Benjamini-Hochberg procedure with a false discovery rate of 10%. A corrected p-value <0.05 was considered to be statistically significant.

Univariate associations between each SNP and t-, f-, b-25OHD, and DBP levels were analyzed using one-way ANOVA. Those SNPs with a significant univariate association were further investigated in a multivariate linear model including their main effects and all possible two-way interactions with vitamin D and DBP levels as outcomes adjusted for the effects of sex and BMI. To improve interpretation, non-significant terms (SNPs and SNP by SNP interactions) were removed from the final model to reach the most parsimonious equation.

Analyses were conducted using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp. Released 2012 Armonk, New York, U.S.A.), and Haploview [17].

3. Results

A total of 892 participants were enrolled. Due to missing data on medical and other information history, or unavailable blood samples, 223 of them were excluded. Among the 669 subjects who were investigated in the full survey [13] we further excluded 207 participants who met at least one of the specific exclusion criteria of the present analysis. Thus, the final analytical sample consists of 462 participants. Characteristics of all excluded and included survey participants are shown in Table 1. No difference was noted between the excluded and included population regarding age, anthropometric data. A male to female ratio was slightly higher, total-, free- and biologically available vitamin D levels were significantly lower in included subjects than in the excluded survey population. Vitamin D binding protein levels were similar in these groups. Females exhibited slightly lower t- and f-25OHD levels than males in both populations (13.9±6.3 vs. 15.6±7.5, and 8.7±4.7 vs. 10.2±5.2, respectively). BMI was not related to t- or f-25OHD levels in the currently analyzed population similarly to our previous finding in the full survey population [13]. All investigated SNPs fulfilled the criteria of Hardy-Weinberg

equilibrium. Furthermore, the SNPs studied here were not in linkage disequilibrium, with the exceptions of rs7041 and rs4588.

Table 2 shows unadjusted associations between investigated SNPs and levels of t-, f-25OHD and DBP. We found significant associations between rs7935125 (*NADSYN1*), rs222054, rs7041 (*GC*), rs2762939, rs2762941, and rs2209314 (*CYP24A1*) polymorphisms and t-25OHD levels. DBP levels were associated with rs2209314 (*CYP24A1*), rs17467825 and rs4588 (*GC*) polymorphisms. None of the other SNPs were related to t-25OHD or DBP.

As b-25OHD and f-25OHD were extremely tightly correlated ($r=0.98$, $p<0.0001$), we only present results for f-25OHD in the text but findings are also applicable for b-25OHD. Free 25OHD levels were not related to any SNPs after Benjamini-Hochberg correction. (Table 2)

All six SNPs with a univariately significant association with t-25OHD were entered into one multivariable linear model that was further adjusted for age, sex and BMI. Two of these SNPs (rs2762939 and rs2209314) were removed from the model as they did not improve model fit. In the final model we also retained an interaction term between rs7935125 (*NADSYN1*) and rs2762941 (*CYP24A1*). According to the calculated standardized effect sizes, the four selected SNPs explained 13.1% of the variance in t-25OHD levels. (Table 3) In the present study, more than half of the genetic effects (7.8 of 13.1%) of the variance in t-25OHD levels were linked to the rs7935125 (*NADSYN1*) and rs2762941 (*CYP24A1*) polymorphisms. When comparing the most favourable haplotype of the two novel SNPs (rs2762941 – GG, rs7935125 – AA) with the most adverse haplotype (rs2762941 – AA, rs7935125 – CC) the observed difference in the mean t-25OHD level is quite considerable (15.32 vs. 8.08 ng/ml, respectively).

4. Discussion

The present investigation based on a nationally representative population provides preliminary information on the genetic determinants of total-, free-, and biologically available vitamin D

levels by minimizing environmental and other non-genetic influences on these levels by study design. The investigated SNPs explain 13.1% of the variance in t-25OHD level. *GC* polymorphisms were related to total- but not the f-25OHD or b-25OHD levels. We report two novel SNPs (in *NADSYN1* and *CYP24A1* gene) that showed a weak association with f-25OHD levels, but the association was no longer significant after Benjamini-Hochberg correction. The genetic effect was only evincible on t-25OHD levels reaching an R^2 of 13%.

In previous studies estimating the genetic influence on t-25OHD levels had varying results from as low as 1-4% to as high as 77%. In general, GWA studies reported very low inheritability (only 1-4%) [6, 7], while twin studies (Twins UK study – 43%, Canadian twin cohort – 77%) reported much larger genetic effects [18, 19] Similarly to these twin studies, linkage studies demonstrated substantially larger heritability (28-80%) [8, 9]. Apart from the inherent differences in methodology, the variable results may be attributable to the relative weight of environmental and lifestyle factors related to vitamin D levels.

In recent years, several studies have evaluated the association between the variations of genes encoding proteins involved in vitamin D metabolism and serum t-25OHD [20-22] levels with inconclusive results. Some studies reported strong correlations [6, 7], while some were unable to prove such association [23]. Similarly to the above reasoning, a possible cause of unequivocal results might be related to the noise related to uncontrolled environmental factors. Furthermore, other confounders, such as population composition related to ethnicity, specific diseases [9, 24] or age distribution [2] could have also decreased observed effects.

In the present study, the genetic effect on t-25OHD was smaller than that of twin observations but substantially higher than those in earlier GWA studies. Compared to the large-scale GWA studies, the present survey investigated SNPs that are known to be involved in vitamin D metabolism (hypothesis driven analysis) and by restricting the timing of sample collection and

by several exclusions minimized environmental and other non-endogenous effects on vitamin D levels.

We report two novel SNPs rs7935125 (*NADSYN1*) and rs2762941 (*CYP24A1*) with an effect on t-25OHD levels which had not been previously reported. These SNPs are located in non-coding regions, and thus have no direct effect on amino acid sequence, but may alter the activity of transcription factor binding affinity. This hypothesis is supported by the fact that rs7935125 is in the binding region of the Ikaros family of zinc finger proteins-1 (*IKZF1*) transcription factor and rs2762941 is in the area of several transcription factors' binding regions (*BCL3*, *MAFF*, *MAFK*, and *SIN3A*) [25].

It is also possible that rs7935125 is in linkage disequilibrium with rs12785878 (an SNP found to be related to t-25OHD levels in a GWA study) [6, 25], although this is also an intronic SNP.

We also report a previously unknown association between rs222054 (*GC*) and t-25OHD levels. This SNP was investigated in a previous study, but as it did not fulfill the criteria of the Hardy-Weinberg equilibrium, it was excluded there from further analysis [10]. This is an intronic SNP, but no detailed information could be found in the literature about its function or whether it is in linkage with other polymorphisms.

The fourth SNP that demonstrated significant association with t-25OHD levels was rs7041 (*GC*). This is a well-known polymorphism of the vitamin D binding protein that was shown to be associated with circulating t-25OHD levels [26-28] The present study is confirmatory in regard to this SNP.

In addition to rs27629411, two other *CYP24A1* SNPs (rs2762939 and rs2209314) showed univariate associations with t-25OHD levels but it became non-significant after adjusting for BMI, age, and sex. Barry et al. [29] demonstrated an association between vitamin D levels and rs2762939 genotype in healthy, non-Hispanic adults however in that study the confounding effect of BMI was not taken into account leading to somewhat different conclusions [13].

The authors of the present report also investigated genetic determinants of serum DBP levels. We confirmed the role of rs4588 and found a non-significant association between rs7041 and DBP levels. [30, 31] We also report statistically significant associations between rs17467825 and rs2209314 and DBP levels. Rs17467825 is located in the 3′-UTR region of the *GC* gene and is in strong linkage disequilibrium with causal functional variants affecting the mRNA stability of DBP [32]. Song W. et al. reported that this SNP is in a haplotype block with rs7041 and rs4588 and affected DBP levels in a periodontitis patient group [31].

Our findings confirm the importance of rs4588 and rs17467825 on DBP metabolism, but we were unable to demonstrate an effect on t-25OHD levels despite the fact that earlier studies identified such associations in different ethnic groups [33, 34].

There is only limited information about genetic determinants of free- or biologically available 25OHD levels. Yao et al. investigated a set of SNPs in a hypothesis driven analysis however, none of the included SNPs showed such an association [35]. Similarly, we were unable to show significant association between the f-25OHD level and the investigated SNPs. The genetic effect on f-25OHD proved to be far less than that was for t-25OHD. To our surprise the SNPs associated with DBP levels were unrelated to f-25OHD levels in our population.

The present study has certain limitations. First, it is a cross-sectional study. Thus, alteration of the genetic effect on the t-25OHD levels after vitamin D treatment or UV-B radiation was not evaluated. Second, we did not measure but calculated free- and bioavailable 25OHD levels, because the direct assay was not available at the time of analyses. Measuring this parameter could strengthen our conclusion, however, the calculated and measured f- and b-25OHD levels strongly correlated in an earlier study [15] suggesting that calculated measures are highly reliable.

Third, we did not incorporate the *GC* genotypes in the calculation of f-25OHD. Instead we used the widely accepted Bikle's formula [15], which incorporates only DBP and serum albumin

concentration in the equation however does take into account the genetic variations in DBP affinity. While it is plausible that the Chun method which also includes the *GC* genotype could be more precise [36], it is more likely to be of particular importance in ethnically mixed populations where the six allelic combinations occur at different frequencies [37] and different allelic forms circulate at varying concentrations [38].

In the present study we decided not to use the Chun's method because (1) we analyzed data from an ethnically homogenous population and (2) we wanted to be consistent with our previous publication on the same population that was written before any genetic data was available [13]. Furthermore, we think that by using Bikle's instead of Chun's equation for the f-25OHD calculation we only introduced a limited bias as the t-25OHD level was extremely low and the f-25OHD values generated by the Chun's „eSS models" were consistent with Bikle's in previous reports [36, 39].

Finally, not all genes and not all SNPs in the genes of the 25OHD pathway were investigated. The *CYP3A4*, *CYP27A1* and *CYP27B1* genes may be related to serum 25OHD levels however were not examined in the current paper. The investigators selected SNPs based on previous evidence, and also included promising targets based on the available data.

The strengths of our survey were the nation-wide representative population, vitamin D levels being free of environmental factors, and the investigation of the classic metabolic pathway of 25OHD metabolism.

5. Conclusion

In conclusion, the present study report of two previously uninvestigated SNPs (rs7935125 of *NADSYN1* and rs2762941 of *CYP24A1*) that are responsible for more than half of the genetic effect on t-25OHD levels. None of the investigated SNP was associated with f-25OHD level. These outcomes suggest that t-25OHD levels are significantly influenced by genetic factors

through *NADSYN1*, *CYP24A1*, and *GC* polymorphisms. However, the clinical significance of this remains speculative, since f-25OHD levels show only a very small genetic determination.

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7. Disclosures

The trial was approved by the Scientific and Research Ethics Committee of the Medical Research Council of Hungary and was carried out in compliance with the World Medical Association Declaration of Helsinki's Ethical Principles for Medical Research Involving Human Subjects.

7.1. Declaration of interest

The authors declare that they have no conflicts of interest to disclose.

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Table 1. Subject characteristics of study population and excluded subjects.

Parameter	Study population (n=462)	Excluded subjects (n=207)	p-value
Age (years ± SD)	49.55 ± 16.63	49.70±15.44	0.108
Height (m ± SD)	1.70 ± 0.95	1.70±0.09	0.367
Weight (kg ± SD)	75.7 ± 15.9	76.3±17.3	0.258
Body mass index (kg/m^2 ± SD)	25.97 ± 4.5	26.13±4.96	0.354
Sex (n (%))			
Male	228 (49%)	86 (42%)	0.066
Female	234 (51%)	121 (58%)	
total 25-OH-vitamin-D (nmol/l ± SD)	14.73±6.96	20.38±9.55	<0.001*
bioavailable 25-OH-vitamin-D (nmol/l ± SD)	3.89±2.05	7.02±6.72	<0.001*
free 25-OH-vitamin-D (pmol/l ± SD)	9.52±5.00	17.34±16.08	<0.001*
Vitamin D binding protein (mg/l ± SD)	309.2±84.4	285.9±129.8	0.007*
PTH (ng/l ± SD)	26.99±18.03	22.58±10.37	0.034*

Differences across study population and excluded subject were assessed by independent sample t-tests for continuous and with Chi-square tests for categorical variables.

Table 2. Total-, free-, bioavailable 25-OH-vitamin-D, and vitamin D binding protein levels in subjects with different genotypes.

		Allele		t-25OHD (ng/ml)			*p*	DBP (mg/l)			*p*	f-25OHD (pmol/l)			*p*	b-25OHD (nmol/l)			*p*
Gene	*SNP*	*ref.*	*var.*	*reference homozygous*	*heterozygous*	*variant homozygous*	*ref. vs. var. homozygous*	*reference homozygous*	*heterozygous*	*variant homozygous*	*ref. vs. var. homozygous*	*reference homozygous*	*heterozygous*	*variant homozygous*	*ref. vs. var. homozygous*	*reference homozygous*	*heterozygous*	*variant homozygous*	*ref. vs. var. homozygous*
NADSYN1	rs7935125	C	A	**12.2**	**14.2**	**16.0***	***0.003****	310.4	307.7	310.6	*1.000*	**7.7**	**9.2**	**10.2**	***0.012***	**3.2**	**3.8**	**4.1**	***0.021***
GC	rs17467825	A	G	15.9	13.7	14.1	*0.357*	**318.9**	**306.3**	**278.0***	***0.007****	9.8	9.0	10.2	*1.000*	4.0	3.7	4.1	*1.000*
	rs222054	C	G	**13.9**	**15.6**	**18.0***	***0.026****	300.1	319.6	325.1	*0.400*	9.1	9.8	10.6	*0.424*	3.7	4.0	4.6	*0.216*
	rs4588	G	T	15.9	13.8	14.2	*0.344*	**320.2**	**305.5**	**277.3***	***0.004****	9.8	9.0	10.2	*1.000*	4.0	3.7	4.2	*1.000*
	rs7041	A	C	**13.6**	**14.6**	**16.2***	***0.006****	300.1	304.2	324.7	*0.082*	9.1	9.4	9.9	*0.759*	3.8	3.9	4.0	*0.759*
CYP2R1	rs1993116	A	G	14.6	14.8	15.0	*1.000*	287.6	313.2	313.6	*1.000*	10.0	9.2	9.5	*0.085*	4.0	3.8	3.9	*1.000*
	rs10500804	T	G	14.7	14.9	14.9	*1.000*	301.6	311.2	317.1	*0.539*	9.7	9.4	9.5	*1.000*	3.9	3.9	3.8	*1.000*
	rs11023374	T	C	14.9	14.5	16.6	*0.477*	306.6	307.0	322.9	*0.811*	9.7	9.2	10.2	*1.000*	3.9	3.8	4.2	*1.000*
CYP24A1	rs4809959	A	G	15.2	14.6	15.0	*1.000*	293.5	314.4	313.3	*0.260*	10.3	9.3	9.2	*0.250*	4.3	3.8	3.8	*0.206*
	rs927650	T	C	15.4	14.8	14.4	*0.481*	305.5	308.7	313.2	*1.000*	9.9	9.4	9.2	*0.814*	4.1	3.8	3.8	*0.448*
	rs4809960	T	C	14.7	15.1	13.2	*0.822*	301.4	321.2	299.5	*1.000*	9.9	9.0	8.8	*1.000*	4.0	3.8	3.6	*1.000*
	rs2209314	T	C	**15.6**	**13.6**	**13.1***	***0.017****	**306.2**	**309.8**	**360.9**	***0.036***	**9.9**	**8.9**	**5.8**	***0.012***	**4.1**	**3.6**	**3.1**	***0.008***
	rs6022999	A	G	15.1	14.4	16.4	*1.000*	306.1	313.6	311.6	*1.000*	9.6	9.3	10.4	*1.000*	3.9	3.8	4.3	*1.000*
	rs2181874	G	A	15.0	14.5	14.5	*1.000*	310.8	304.4	326.2	*1.000*	9.5	9.5	9.0	*1.000*	3.9	4.0	3.6	*1.000*
	rs2585428	C	T	14.9	14.7	15.0	*1.000*	299.0	313.6	314.6	*0.505*	9.9	9.4	9.0	*0.695*	4.1	3.8	3.8	*0.575*
	rs3787555	C	A	14.6	15.4	13.1	*0.580*	308.7	309.4	315.4	*1.000*	9.5	9.8	7.8	*0.178*	3.9	4.0	3.2	*0.195*
	rs2762939	G	C	**15.5**	**14.0**	**11.3***	***0.018****	309.8	306.7	321.8	*1.000*	9.8	9.1	8.3	*0.528*	4.0	3.8	3.3	*0.326*
	rs2244719	C	T	15.0	15.6	13.2	*0.070*	310.1	306.5	316.2	*1.000*	9.5	10.1	8.3	*0.195*	3.9	4.1	3.4	*0.144*
	rs2762941	G	A	**15.2**	**15.4**	**11.2***	***0.001****	309.2	310.5	304.3	*1.000*	9.7	9.7	8.1	*0.136*	4.0	4.0	3.3	*0.097*

Univariate associations between each SNP and t-, f-, b-25OHD, and DBP levels were analyzed using one-way ANOVA, Bonferroni post hoc test. **Bold** text highlights where Bonferroni post hoc test's $p<0.05$; * marks differences remained significant after FDR correction

Table 3. Relationship between total 25-OH-vitamin-D levels and significantly associated genotypes

Parameter	B	Std. error	P-value	Explained variance (eta-square)
rs7041	-2.959	0.905	0.005	2.6%
	-1.228	0.718		
rs7935125	-5.787	2.844	0.005	2.7%
	-3.031	2.179		
rs2762941	0.830	1.956	0.006	2.6%
	3.291	1.969		
rs222054	-3.008	1.261	0.004	2.7%
	-1.157	1.266		
rs7935125*rs2762941 interaction	4.966	3.299	0.040	2.5%
	1.195	3.209		
	3.247	2.371		
	-0.697	2.378		
calculated genetic effect			**NA**	**13.1%**
sex	1.817	0.617	0.003	2.2%
BMI	-0.130	0.067	0.053	0.9%
corrected model			0.000	16.7%

Multiple linear regression with t-25OHD as dependent and univariately significantly associated SNPs (rs7041; rs7935125; rs2762941; rs222054), their significant interactions (rs7935125*rs2762941), and sex and BMI as covariates.