Title: The effect of age and gender on the genetic regulation of serum 25-hydroxyvitamin D - the FIN-D2D Population-Based Study.

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Abstract

In addition to sunlight and dietary sources, several genes in the metabolic pathway of vitamin D affect serum 25-hydroxyvitamin D (25OHD) concentration. It is not known whether this genetic regulation is influenced by host characteristics. We investigated the effect of age and gender on the genetic regulation of serum 25OHD concentration.

In total, 2868 Finnish men and women aged 45-74 years participated in FIN-D2D population-based health survey in 2007. Of the 2822 participants that had serum 25OHD concentration available, 2757 were successfully genotyped. Age and gender-dependent association of SNPs with serum 25OHD concentration was studied in 10 SNPs with previously found association with vitamin D metabolites.

Associations of 3 SNPs with serum 25OHD concentration were dependent on age with greater effects on younger (≤60 y) than older (>60y) adults (rs10783219 in *VDR*, rs12512631 in *GC* and rs3794060 in *NADSYN1/DHCR7;* $p_{interaction}$=0.03, 0.02 and 0.01, respectively). The results suggested a novel association between serum 25OHD concentration and rs8082391 in *STAT5B* gene in men but not in women ($p_{interaction}$=0.01). After multiple testing correction with false discovery rate method, two age-dependent interactions (rs3794060 in *NADSYN1/DHCR7* gene and rs12512631 in *GC* gene) remained statistically significant.

This is the first study to suggest that genetic regulation of serum 25OHD concentration is age-dependent. Our results also indicated a novel association between serum 25OHD concentration and SNP in *STAT5B* gene in men. The results need to be confirmed in future studies preferably in a larger sample.

Keywords: Vitamin D, 25-hydroxyvitamin D, single nucleotide polymorphism, genetic regulation, population-based study, vitamin D receptor

Abbreviations:

25OHD	25-hydroxyvitamin D
BMI	Body mass index
DBP	Vitamin D binding protein
DHCR7	7-dehydrocholesterol reductase
GC	Group-specific component
NADSYN1	NAD synthetase 1
VDR	Vitamin D receptor

1. INTRODUCTION

Vitamin D is a prohormone that is produced in the skin through sunlight and obtained from dietary sources. The hormonally active form of vitamin D, 1,25-hydroxyvitamin D (1,25OHD), regulates calcium and phosphate absorption and bone metabolism [1]. 1,25-OHD has also important immunomodulatory properties [2].

The vitamin D status of a person is commonly estimated by measuring serum 25-hydroxyvitamin D (25OHD) concentration. 25OHD is the main vitamin D metabolite in the circulation and is the main source for the short-lived active form of vitamin D.

Serum 25OHD concentration depends on the exposure to UV radiation, on the intake of vitamin D from the diet and supplements [1] as well as on physiological and life-style factors, such as age, body mass index (BMI), smoking and physical activity [3,4]. Also genetic factors affect serum 25OHD concentration. The heritability of serum 25OHD concentration has been estimated to be at least 30% [5,6] and is conferred mainly in single nucleotide polymorphisms (SNPs) in genes in the metabolic pathway of vitamin D [7-13].

The metabolic pathway of vitamin D consists of several steps. Vitamin D is synthesized from 7-hydrocholesterol in the skin. *NADSYN1/DHCR7* locus controls the availability of 7-dehydrocholesterol by converting it to cholesterol [14]. Vitamin D is converted to 25OHD mainly by *CYP2R1* and to some extent by *CYP27A1*. In the circulation 25OHD binds to the vitamin D binding protein (DBP) (encoded by the group-specific component *GC* gene). Cubilin (encoded by the cubilin gene *CUBN*) together with megalin facilitates the renal cell uptake of the 25OHD–DBP complex. The loss of a functional cubilin results in decrease in 25OHD and 1,25OHD levels in the blood [15]. 25OHD is hydroxylated to 1,25OHD by 1α-hydroxylase (encoded by *CYP27B1*). The active form of vitamin D acts through the intracellular transcription factor vitamin D receptor (*VDR*) [16].

Megalin and cubilin, endocytic receptors in proximal tubule cells, are involved in the reabsorption of vitamin D binding protein from glomerular filtrates and the subsequent intracellular conversion of 25-hydroxyvitamin D_3 to biologically active 1α,25-dihydroxyvitamin D_3.

Single nucleotide polymorphisms (SNPs) associate with several diseases and with physiological traits. Some of these associations may be missed in genome-wide association studies (GWAS), since the strength of the associations can vary between different subgroups [17,18]. The most well-known factors that modify the associations of SNPs with an outcome are age and gender. For example, the associations between several SNPs and waist-to-hip-ratio, waist circumference and lipid traits have shown to be different between women and men [19-21]. Most of these associations have shown to be stronger in women than in men and have been suggested to relate with the action of sex hormones. Age has shown to modify associations between SNPs and BMI with stronger associations in younger than older adults [22-24].

Our aim was to study whether age and gender affect the associations between serum 25OHD concentration and SNPs in the metabolic pathway of vitamin D.

2. MATERIALS AND METHODS

2.1 Study population

A detailed description of the study population has been previously published [25,26]. Briefly, this study is a part of the FIN-D2D population-based health survey 2007, which is a joint project within the Finnish National Diabetes Prevention program FIN-D2D. Initially, a random sample of 4500 people aged 45-74 years, stratified according to gender, 10-year age groups (45-54, 55-64, and 65-74

years), and geographical areas (Hospital Districts of Pirkanmaa, Central Finland and South Ostrobothnia), was selected from the National Population Register in August 2007. Serum samples of all participants were collected during October, November and December 2007.

A total of 2868 persons (64%) participated in the health examination during October, November and December in 2007. Of these, serum 25OHD concentration was available of 2822 persons [3]. Of the 2822 persons, 2785 had a DNA sample available. Genotyping failed (no result in any of the SNPs studied) in 28 samples, resulting in the final number of 2757 genotyped samples. Written informed consent was collected from all participants. The Ethics Committee of the Helsinki and Uusimaa Hospital District approved the study.

2.2 Vitamin D analyses

Serum 25OHD concentration measurement in this cohort has been previously described [3]. Briefly, serum 25OHD concentration was determined by chemiluminescent microparticle immunoassay by Architect *i* system (Abbott Laboratories, Abbott Park, IL, USA). The analytical reliability of 25OHD assay was assured by participation in the vitamin D External Quality Assessment Scheme i.e. DEQAS (Charing Cross Hospital, London UK). The bias compared to all-laboratory trimmed mean in the DEQAS was −6.4%±7.1 (mean ± SD).

2.3 DNA extraction, SNP selection and genotyping

Genomic DNA was extracted from whole blood using an automated Chemagen DNA extraction equipment (PerkinElmer, Waltham, MA) or using a QIAamp DNA Blood Maxi Kit (QIAGEN GmbH, Hilden, Germany) following the protocol of the kit with slight modifications. A total of 13 SNPs in 7 candidate genes were initially selected on basis of evidence of associations with serum 25OHD concentration in previously published studies [7-13]. SNPs in *STAT5B* gene, which is a member of signal transducers and activators of transcription (STATs), was selected on basis of previously found associations between 1,25OHD, *VDR* and *STAT5* [27,28].

Genotyping was done using TaqMan (Applied Biosystems, Paisley, United Kingdom). One SNP (rs7975232) in the *VDR* gene was not in Hardy-Weinberg equilibrium and was excluded from further analyses. SNPs in the *CYP27B1* gene (rs10877012 and rs703842) and SNPs in the *STAT5B* gene (rs8082391 and rs8064638) were in strong linkage disequilibrium and had identical results. Thus, the results of only one SNP of these SNP groups are presented. The final number of SNPs was therefore 10.

2.4 Statistical analyses

Associations between SNPs and serum 25OHD concentrations were evaluated with linear regression models. The analyses were adjusted for BMI, age, smoking, vitamin D supplement use, physical activity, month of sample collection, blood glucose and triglycerides. An interaction term was included in the models to evaluate possible interaction with age / gender. Multiple testing was controlled for using the false discovery rate (FDR) method (a step-up procedure described by Benjamini and Hochberg [29], using 0.1 as the criterion). If the original *p* value was smaller than the Benjamini–Hochberg critical value, the interaction was considered statistically significant. Statistical analyses were carried out using Stata statistical package 12.1 (Stata-Corp. 2011, Stata Statistical Software: Release 12, College Station, TX, USA; StataCorp LP).

3. RESULTS

Mean serum 25OHD concentration of the study population, as well as serum 25OHD separately for women and men, is presented in table 1. Also, serum 25OHD concentration of participants that used or did not use vitamin D containing supplements, is present in table 1.

Table 1. Serum 25OHD concentrations in Finnish population aged 45-74 years.

	All (n)	**Men (n)**	**Women (n)**	**Participants that use vitamin D supplements (n)**	**Participants that do not use vitamin D supplements (n)**
Serum 25OHD (nmol/l)	57.6 (2822)	58.2 (1348)	57.1 (1474)	62.9 (788)	55.6 (2034)

The association between 10 SNPs and serum 25OHD concentration in younger (≤60y) and older (>60y) adults, and in women and men, was studied in 2757 persons.

Association of 3 SNPs with serum 25OHD concentration varied according to age with stronger associations in younger than older adults (rs10783219 in the *VDR* gene, rs12512631 in the *GC* gene and rs3794060 in the *NADSYN1/DHCR7* gene locus; $p_{interaction}$=0.03, 0.02 and 0.01, respectively) (table 2). Age-dependent interactions in the *GC* gene and in the *NADSYN1/DHCR7* gene locus (figure 1) remained statistically significant after adjusting for multiple comparisons with FDR method.

Table 2. Association of serum 25-hydroxyvitamin D concentration with SNPs in younger (≤60y; n=1425) and older (>60y; n=1332) adults.

SNP	Gene	*p* Younger adults (≤60y) (n)	*p* Older adults (>60y) (n)	*p* (interaction)[a]	Benjamini-Hochberg critical value
rs731236	*VDR*	0.073 (1406)	0.30 (1319)	0.07	
rs4516035	*VDR*	0.013 (1407)	0.14 (1320)	0.27	
rs10783219	*VDR*	0.061 (1406)	0.075 (1320)	**0.039**	**0.03**
rs12512631	*GC*	0.051 (1402)	0.18 (1316)	**0.018**[b]	**0.02**
rs17470271	*CYP27A1*	0.59 (1402)	0.22 (1319)	0.87	
rs703842	*CYP27B1*	0.97 (1402)	0.49 (1320)	0.60	
rs3794060	*NADSYN1 /DHCR7*	<0.001 (1407)	0.49 (1321)	**0.006**[b]	**0.01**
rs8082391	*STAT5B*	0.88 (1407)	0.32 (1320)	0.56	
rs1801222	*CUBN*	0.11 (1406)	0.15 (1322)	0.31	
rs12766939	*CUBN*	0.59 (1406)	0.76 (1311)	0.84	

[a] Analyses were adjusted for BMI, smoking, vitamin D supplement use, physical activity, month of sample collection, blood glucose and triglycerides. Interaction refers to age.

[b]Statistically significant after multiple testing correction using false discovery rate. If the original *p* value was smaller than the Benjamini–Hochberg critical value, the interaction was considered statistically significant.

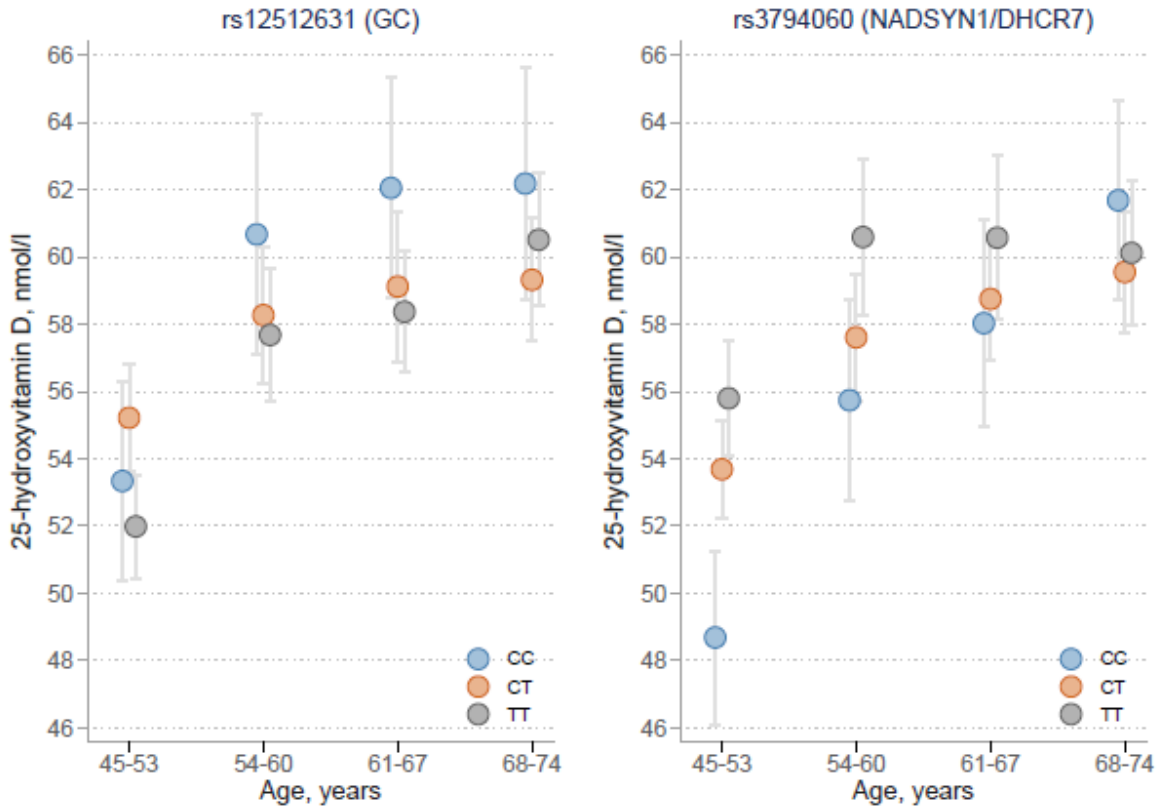

Figure 1. Age-dependent association between serum 25-hydroxyvitamin D concentration and SNPs. The associations between serum 25OHD concentration and rs12512631 in *GC* gene and rs3794060 in *NADSYN1/DHCR7* gene were stronger in younger than older adults.

One SNP (rs731236) in the *VDR* gene showed an age-dependent association with serum 25OHD concentration ($p_{interaction}$=0.01) before adjusted for the confounding factors (BMI, smoking, vitamin D supplement use, physical activity, month of sample collection, blood glucose and triglycerides), but this interaction did not remain statistically significant after the adjustment for these factors (p=0.07).

A novel association between rs8082391in *STAT5B* gene with serum 25OHD concentration in men was found (p=0.008; table 3). The association was not seen in women ($p_{interaction}$=0.01; table 2). The gender-dependent interaction did not remain statistically significant after multiple testing correction with FDR method.

Table 3. Association of serum 25OHD concentration with SNPs in women and men.

SNP	Gene	*p* Women (n)	*p* Men (n)	*p* (interaction)[a]	Benjamini-Hochberg critical value
rs731236	*VDR*	0.67 (1423)	0.96 (1302)	0.83	
rs4516035	*VDR*	0.64 (1426)	0.21 (1301)	0.80	
rs10783219	*VDR*	0.29 (1427)	0.34 (1299)	0.17	
rs12512631	*GC*	0.15 (1419)	0.16 (1299)	0.47	
rs17470271	*CYP27A1*	0.26 (1423)	0.95 (1298)	0.70	
rs703842	*CYP27B1*	0.18 (1420)	0.58 (1302)	0.15	
rs3794060	*NADSYN1/DHCR7*	0.03 (1429)	0.006 (1299)	0.51	
rs8082391	*STAT5B*	0.44 (1423)	0.008 (1304)	**0.01**	**0.01**
rs1801222	*CUBN*	0.24 (1426)	0.10 (1302)	0.84	
rs12766939	*CUBN*	1.00 (1422)	0.15 (1295)	0.39	

[a]Analyses were adjusted for BMI, age, smoking, vitamin D supplement use, physical activity, month of sample collection, blood glucose and triglycerides. Interaction refers to sex.

4. DISCUSSION

We found that the strength of associations between serum 25OHD concentration and SNPs in the metabolic pathway of vitamin D are age-dependent with stronger associations in younger (≤60y) than older (>60y) adults. However, the present study being the first study to report this results, as well as being a small study for a genetic study, this will need to be confirmed in a larger sample. Our results also indicate an association between serum 25OHD concentration and a SNP in *STAT5B* gene in men but not in women. According to our knowledge, this SNP in *STAT5B* gene has never been associated with serum 25OHD concentration before. In our study, the gender-dependent interaction did not, however, remain statistically significant after multiple testing correction.

Strength of our study was the well-defined study population of the FIN-D2D population-based health survey 2007. We had the possibility to adjust the analyses for several vitamin D status-related physiological and life-style factors which is rarely possible in genetic studies. These factors were seen to modify results: one initially statistically significant age-dependent interaction between serum 25OHD concentration and rs731236 in the *VDR* gene disappeared when the analyses were adjusted for vitamin D status-related factors (BMI, age, smoking, vitamin D supplement use, physical activity, month of sample collection, blood glucose and triglycerides).

To our knowledge, there are no previous studies on the factors that modify the genetic component of the vitamin D status. However, several previous studies have shown that age and gender modify the associations between SNPs and an outcome, for example adiposity distribution and BMI [19-24]. In addition, it has been shown that an environmental factor can change the direction of an association between SNP and outcome: a SNP in *CHRNA5-A3-B4* gene cluster has been shown to associate with a lower BMI in current smokers and with higher BMI in never smokers [17].

Several physiological and biological changes take place when people get older, and thus age can serve as a marker of a variety of host characteristics. In our previous work of the FIN-D2D population-based study we found that the mean serum 25OHD concentration is higher among older than younger adults [3]. Based on the present results, it seems that at least partly this may be explained by the fact that age affects the genetic component of the serum 25OHD concentration.

The age range in our study population was limited consisting of 45 to 74-year-olds. It is possible that larger differences exist in the genetic regulation of serum 25OHD concentration for example between children and adults. This will need to be clarified in the future studies in more detail.

Our results indicate a novel association between serum 25OHD concentration and rs8082391 in *STAT5B* gene in men. Previously it has been found that active vitamin D may exert an anti-inflammatory action by influencing the crosstalk between STAT5 and the *VDR* [28]. VDR-STAT5 complexes prevent inflammation by affecting the cytokine profile [28]. *STAT5B* has been shown to have a central role in the adaptive immune response and to mediate sex-dependent effects of the growth hormone [30-32]. *STAT5B* SNP included in the present study (rs8082391) has been suggested to affect *STAT5B* transcription levels [33]. The known sex-dependent effects of *STAT5B* gene [30] may at least partly explain the fact that in the present study the association between serum 25OHD concentrations and SNP in *STAT5B* gene was only seen in men.

This study provides novel insight to the genetic regulation of serum 25OHD concentration. Our results suggest for the first time that there is an age-dependent effect of certain vitamin D pathway SNPs on the serum 25OHD concentration. GWAS have identified a large number of genetic variants that associate with several diseases and with physiological traits. Since these associations are influenced by environmental factors, it would be important stratify the study populations in these analyses according to relevant environmental factors and host characteristics. It is likely that in the near future plenty of new environmental, life-style and clinical factors affecting associations between SNPs and serum 25OHD concentration will be identified.

Conclusions

This study suggests for the first time that the genetic regulation of vitamin D status through genes located in the metabolic pathway of vitamin D, is weaker among older than younger adults. The result needs to be, however, confirmed in a larger sample.

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