

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

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36 **Abstract**

37

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

42

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

47

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

55

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

60

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

63

64 **Abbreviations:**

65	25OHD	25-hydroxyvitamin D
66	BMI	Body mass index
67	DBP	Vitamin D binding protein
68	DHCR7	7-dehydrocholesterol reductase
69	GC	Group-specific component
70	NADSYN1	NAD synthetase 1
71	VDR	Vitamin D receptor

72 1. INTRODUCTION

73  
74 Vitamin D is a prohormone that is produced in the skin through sunlight and obtained from dietary  
75 sources. The hormonally active form of vitamin D, 1,25-hydroxyvitamin D (1,25OHD), regulates  
76 calcium and phosphate absorption and bone metabolism [1]. 1,25-OHD has also important  
77 immunomodulatory properties [2].

78  
79 The vitamin D status of a person is commonly estimated by measuring serum 25-hydroxyvitamin D  
80 (25OHD) concentration. 25OHD is the main vitamin D metabolite in the circulation and is the main  
81 source for the short-lived active form of vitamin D.

82  
83 Serum 25OHD concentration depends on the exposure to UV radiation, on the intake of vitamin D  
84 from the diet and supplements [1] as well as on physiological and life-style factors, such as age, body  
85 mass index (BMI), smoking and physical activity [3,4]. Also genetic factors affect serum 25OHD  
86 concentration. The heritability of serum 25OHD concentration has been estimated to be at least 30%  
87 [5,6] and is conferred mainly in single nucleotide polymorphisms (SNPs) in genes in the metabolic  
88 pathway of vitamin D [7-13].

89  
90 The metabolic pathway of vitamin D consists of several steps. Vitamin D is synthesized from 7-  
91 hydrocholesterol in the skin. *NADSYN1/DHCR7* locus controls the availability of 7-  
92 dehydrocholesterol by converting it to cholesterol [14]. Vitamin D is converted to 25OHD mainly by  
93 *CYP2R1* and to some extent by *CYP27A1*. In the circulation 25OHD binds to the vitamin D binding  
94 protein (DBP) (encoded by the group-specific component *GC* gene). Cubilin (encoded by the cubilin  
95 gene *CUBN*) together with megalin facilitates the renal cell uptake of the 25OHD–DBP complex. The  
96 loss of a functional cubilin results in decrease in 25OHD and 1,25OHD levels in the blood [15].  
97 25OHD is hydroxylated to 1,25OHD by 1 $\alpha$ -hydroxylase (encoded by *CYP27B1*). The active form of  
98 vitamin D acts through the intracellular transcription factor vitamin D receptor (*VDR*) [16].

99  
100 Megalin and cubilin, endocytic receptors in proximal tubule cells, are involved in the reabsorption of  
101 vitamin D binding protein from glomerular filtrates and the subsequent intracellular conversion of 25-  
102 hydroxyvitamin D<sub>3</sub> to biologically active 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>.

103  
104 Single nucleotide polymorphisms (SNPs) associate with several diseases and with physiological traits.  
105 Some of these associations may be missed in genome-wide association studies (GWAS), since the  
106 strength of the associations can vary between different subgroups [17,18]. The most well-known  
107 factors that modify the associations of SNPs with an outcome are age and gender. For example, the  
108 associations between several SNPs and waist-to-hip-ratio, waist circumference and lipid traits have  
109 shown to be different between women and men [19-21]. Most of these associations have shown to be  
110 stronger in women than in men and have been suggested to relate with the action of sex hormones.  
111 Age has shown to modify associations between SNPs and BMI with stronger associations in younger  
112 than older adults [22-24].

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

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119 2. MATERIALS AND METHODS

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121 2.1 *Study population*

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

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

130

131 A total of 2868 persons (64%) participated in the health examination during October, November and  
132 December in 2007. Of these, serum 25OHD concentration was available of 2822 persons [3]. Of the  
133 2822 persons, 2785 had a DNA sample available. Genotyping failed (no result in any of the SNPs  
134 studied) in 28 samples, resulting in the final number of 2757 genotyped samples. Written informed  
135 consent was collected from all participants. The Ethics Committee of the Helsinki and Uusimaa  
136 Hospital District approved the study.

137

## 138 2.2 Vitamin D analyses

139

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

146

## 147 2.3 DNA extraction, SNP selection and genotyping

148

149 Genomic DNA was extracted from whole blood using an automated Chemagen DNA extraction  
150 equipment (PerkinElmer, Waltham, MA) or using a QIAamp DNA Blood Maxi Kit (QIAGEN  
151 GmbH, Hilden, Germany) following the protocol of the kit with slight modifications. A total of 13  
152 SNPs in 7 candidate genes were initially selected on basis of evidence of associations with serum  
153 25OHD concentration in previously published studies [7-13]. SNPs in *STAT5B* gene, which is a  
154 member of signal transducers and activators of transcription (STATs), was selected on basis of  
155 previously found associations between 1,25OHD, *VDR* and *STAT5* [27,28].

156

157 Genotyping was done using TaqMan (Applied Biosystems, Paisley, United Kingdom). One SNP  
158 (rs7975232) in the *VDR* gene was not in Hardy-Weinberg equilibrium and was excluded from further  
159 analyses. SNPs in the *CYP27B1* gene (rs10877012 and rs703842) and SNPs in the *STAT5B* gene  
160 (rs8082391 and rs8064638) were in strong linkage disequilibrium and had identical results. Thus, the  
161 results of only one SNP of these SNP groups are presented. The final number of SNPs was therefore  
162 10.

163

## 164 2.4 Statistical analyses

165

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

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## 178 3. RESULTS

179

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

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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)
<b>Serum 25OHD (nmol/l)</b>	57.6 (2822)	58.2 (1348)	57.1 (1474)	62.9 (788)	55.6 (2034)

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The association between 10 SNPs and serum 25OHD concentration in younger ( $\leq 60$ y) and older ( $> 60$ y) 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;  $p_{\text{interaction}}=0.03, 0.02$  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 ( $\leq 60$ y; n=1425) and older ( $> 60$ y; n=1332) adults.

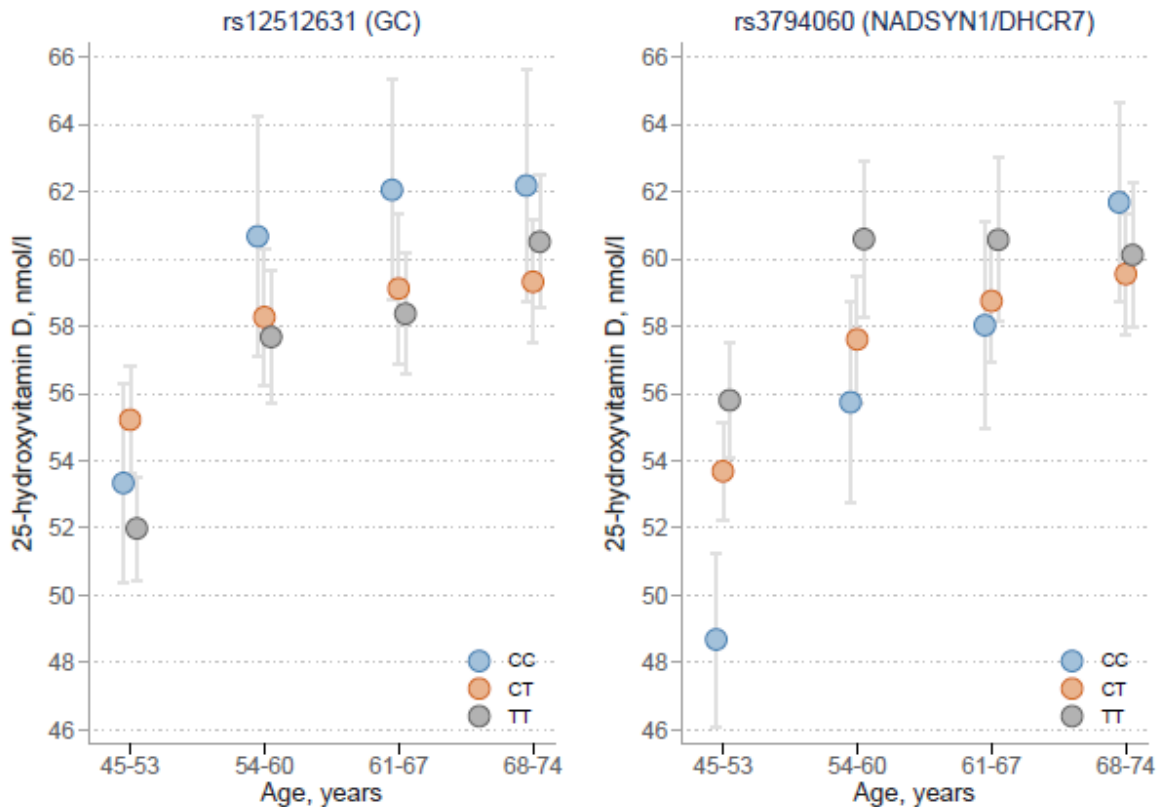
SNP	Gene	$p$ Younger adults ( $\leq 60$ y) (n)	$p$ Older adults ( $> 60$ y) (n)	$p$ (interaction) <sup>a</sup>	Benjamini- Hochberg critical value
rs731236	<i>VDR</i>	0.073 (1406)	0.30 (1319)	0.07	
rs4516035	<i>VDR</i>	0.013 (1407)	0.14 (1320)	0.27	
rs10783219	<i>VDR</i>	0.061 (1406)	0.075 (1320)	<b>0.039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

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

206 <sup>b</sup>Statistically significant after multiple testing correction using false discovery rate. If the original *p*  
 207 value was smaller than the Benjamini–Hochberg critical value, the interaction was considered  
 208 statistically significant.

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213 **Figure 1.** Age-dependent association between serum 25-hydroxyvitamin D concentration and SNPs.  
 214 The associations between serum 25OHD concentration and rs12512631 in *GC* gene and rs3794060 in  
 215 *NADSYN1/DHCR7* gene were stronger in younger than older adults.  
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 218  
 219 One SNP (rs731236) in the *VDR* gene showed an age-dependent association with serum 25OHD  
 220 concentration ( $p_{\text{interaction}}=0.01$ ) before adjusted for the confounding factors (BMI, smoking, vitamin D  
 221 supplement use, physical activity, month of sample collection, blood glucose and triglycerides), but  
 222 this interaction did not remain statistically significant after the adjustment for these factors ( $p=0.07$ ).  
 223  
 224

225 A novel association between rs8082391 in *STAT5B* gene with serum 25OHD concentration in men  
 226 was found ( $p=0.008$ ; table 3). The association was not seen in women ( $p_{\text{interaction}}=0.01$ ; table 2). The  
 227 gender-dependent interaction did not remain statistically significant after multiple testing correction  
 228 with FDR method.  
 229

230  
 231  
 232 **Table 3.** Association of serum 25OHD concentration with SNPs in women and men.

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

<sup>a</sup>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 ( $\leq 60$ y) than older ( $>60$ y) 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].

264  
265 Several physiological and biological changes take place when people get older, and thus age can serve  
266 as a marker of a variety of host characteristics. In our previous work of the FIN-D2D population-  
267 based study we found that the mean serum 25OHD concentration is higher among older than younger  
268 adults [3]. Based on the present results, it seems that at least partly this may be explained by the fact  
269 that age affects the genetic component of the serum 25OHD concentration.

270  
271 The age range in our study population was limited consisting of 45 to 74-year-olds. It is possible that  
272 larger differences exist in the genetic regulation of serum 25OHD concentration for example between  
273 children and adults. This will need to be clarified in the future studies in more detail.

274  
275 Our results indicate a novel association between serum 25OHD concentration and rs8082391 in  
276 *STAT5B* gene in men. Previously it has been found that active vitamin D may exert an anti-  
277 inflammatory action by influencing the crosstalk between *STAT5* and the *VDR* [28]. *VDR-STAT5*  
278 complexes prevent inflammation by affecting the cytokine profile [28]. *STAT5B* has been shown to  
279 have a central role in the adaptive immune response and to mediate sex-dependent effects of the  
280 growth hormone [30-32]. *STAT5B* SNP included in the present study (rs8082391) has been suggested  
281 to affect *STAT5B* transcription levels [33]. The known sex-dependent effects of *STAT5B* gene [30]  
282 may at least partly explain the fact that in the present study the association between serum 25OHD  
283 concentrations and SNP in *STAT5B* gene was only seen in men.

284  
285 This study provides novel insight to the genetic regulation of serum 25OHD concentration. Our  
286 results suggest for the first time that there is an age-dependent effect of certain vitamin D pathway  
287 SNPs on the serum 25OHD concentration. GWAS have identified a large number of genetic variants  
288 that associate with several diseases and with physiological traits. Since these associations are  
289 influenced by environmental factors, it would be important stratify the study populations in these  
290 analyses according to relevant environmental factors and host characteristics. It is likely that in the  
291 near future plenty of new environmental, life-style and clinical factors affecting associations between  
292 SNPs and serum 25OHD concentration will be identified.

### 293 294 *Conclusions*

295  
296 This study suggests for the first time that the genetic regulation of vitamin D status through genes  
297 located in the metabolic pathway of vitamin D, is weaker among older than younger adults. The result  
298 needs to be, however, confirmed in a larger sample.

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301  
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307 decision to submit the article for publication.

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