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Low Vitamin D Levels and Genetic Polymorphism in the Vitamin D Receptor are Associated with Increased Risk of Statin-Induced Myopathy

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Abstract: The main aim of this study was to test the hypothesis whether 25-hydroxyvitamin D (25OHD) levels <50 nmol/L at baseline could predict statin-induced myopathy during the course of treatment. In addition, we analysed the association between a genetic polymorphism in the vitamin D receptor (VDR) and the risk of statin-induced myopathy. We used serum samples from a prospective, observational study in statin-treated patients in Sweden who were thoroughly followed with interviews and questionnaires regarding muscular symptoms (n = 127). In this cohort, 16 developed muscular symptoms and 111 had no muscular symptoms associated with statin treatment during the first year of follow-up. Patients with 25OHD levels <50 nmol/L before starting on statin treatment had four times higher risk of developing muscular symptoms compared with individuals having 25OHD levels >50 nmol/L (RR 4.2; 95% CI 1.7–10.2; $p < 0.01$). The mean levels of 25OHD at baseline were 50 ± 4 nmol/L among patients developing myopathy and 60 ± 2 nmol/L among patients without myopathy ($p < 0.01$). Individuals homozygous for the C allele in the VDR polymorphism *TaqI* (rs731236) had a four times higher risk of developing muscular symptoms; (RR 4.37, 95% CI 1.9–10.1, $p < 0.01$). In conclusion, 25OHD levels <50 nmol/L might be a useful marker to predict muscular adverse events during statin treatment. In addition, the finding that the VDR polymorphism *TaqI* was associated with myopathy may indicate a causal relationship between vitamin D function and myopathy, but larger studies are needed before firm conclusions can be drawn.

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, efficiently reduce cholesterol levels in the blood and have preventive effects on serious cardiovascular events and mortality [1]. Although statins are generally considered as well-tolerated drugs, several reports have demonstrated that statin-induced muscular side effects, such as myalgia, are more common than first demonstrated in clinical trials [2–4].

Vitamin D is synthesized in the skin under influence of UVB light and is further undergoing two hydroxylation steps. The first of these steps occurs in the liver (25-hydroxylation), which produces the proform 25-hydroxyvitamin D (25OHD). The second hydroxylation step generates active vitamin D (1,25 $(OH)_2$ vitamin D) and is catalysed by 1-α hydroxylase (CYP27B1), which is expressed in kidney cells but also in many other cells in the body, including muscle cells [5]. Active vitamin D binds to the vitamin D receptor (VDR) and is transported to the nucleus where it forms a heterodimer with a cognate nuclear receptor partner. The heterodimer complex binds to vitamin D response elements (VDRE) in promoters of several genes, thereby regulating transcription [6]. *In silico* analyses of the human genome have identified approximately 3000 VDRE sequences, but the relevance *in vivo* for the majority of these is unknown [7]. The half-life of 25OHD is about 3 weeks, whereas 1,25-dihydroxyvitamin D only has a half-life of about 4 hr. Generally, systemic levels of the more stable 25OHD are considered to reflect vitamin D status in an individual patient [8]. According to recommendations from the Institute of Medicine in the USA, serum levels below 50 nmol/L are considered to be insufficient [9].

The important role of vitamin D in bone health and calcium homeostasis has been known for a long time. In recent years, it has become evident that vitamin D affects many other cell types, for example in the immune [10], nervous [11] and the cardiovascular systems [12]. In addition, it has been shown that low vitamin D concentrations are associated with increased risk of musculoskeletal pain, [13] especially in patients with fibromyalgia [14]. Interestingly, vitamin D appears to affect muscle strength as it has been shown that old people and patients with a manifest 25OHD deficiency have gained muscle strength when treated with vitamin D [15,16]. In fact, low 25OHD levels have been linked to an increased risk of statin-induced myalgia in two observational studies [17,18]. It has also been reported that repletion of 25OHD levels had a protective affect against myalgia in statin-treated patients [19–21]. However, these studies have not been randomized or placebo-controlled, compromising the validity of the data. Notably, several observational studies fail to establish a relationship between low 25OHD levels and myalgia [22–24]. Thus, the picture is far from complete and there is no consensus in the field.

The human VDR is polymorphic, and previous studies have suggested that the SNP *TaqI* (rs731236) affects the function of

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VDR, probably due to altered expression pattern [25,26]. In addition, we have recently shown that individuals homozygous for the C allele of this SNP had significantly lower expression of VDR in foetal liver tissue [27].

The aim of this study was to test the hypothesis that 25OHD levels <50 nmol/L could predict the risk of statin-induced myopathy. We also investigated the role of the *TaqI* genetic polymorphism in VDR for the risk of developing statin-induced myopathy.

Materials and Methods

Study population. We analysed serum from a previously performed observational clinical study in which 180 patients starting on statin treatment (n = 120) or switching from one statin to another (n = 60) were followed for 1 year regarding onset of muscular symptoms with questionnaires (described in ref. 28 and in Skilving *et al.*, *Manuscript 2015*). The questionnaires included 25 questions covering muscular symptoms, daily life activities, statin compliance, educational level and lifestyle factors, for example alcohol and tobacco use and exercise. There were fixed response alternatives on muscle symptoms and their impact on daily life activities on a four-step categorical scale and on a numerical scale (NRS, 1–10). Blood samples were drawn at baseline and after approximately 2 months of statin treatment and (if possible) every third month up to 1 year after starting treatment. In this study, we used the samples drawn at baseline. The participants filled in the questionnaire about muscular pain at baseline and at every study visit, approximately every third month, up to 1 year after inclusion. Myopathy was defined according to the criteria of the American College of Cardiology (ACC), American Heart Association (AHA) and National Heart, Lung and Blood institute (NHLBI) [29]. Symptoms meeting these criteria were assessed and classified as 'probable' or 'possible', according to WHO. To be classified as a patient 'with myopathy', only patients with 'probable' were included. To be classified as a patient 'without myopathy', only those with neither 'possible' nor 'probable' were included.

From the original study of 180 patients, 127 patients were included in this study (37 were 'statin switchers' and 90 were 'statin naïve'). There were 16 patients who had obvious muscular symptoms that were evaluated to be 'probably caused by statin therapy' according to the WHO criteria. One hundred and eleven (n = 111) patients from the original cohort had no muscular symptoms but could have other adverse reactions. These were defined as 'patients without myopathy'. Patients who had muscular symptoms but where the association with statin therapy was not assured were excluded (n = 15). Patients who were lost to follow-up or where serum samples were missing were excluded (n = 38). All patients with and without myopathy had Creatine Kinase values within the normal range throughout the study. Compliance to treatment was assured, as all patients had decreased LDL levels.

Ethical statement. Written informed consent was obtained from all participants. The study was approved by the Karolinska Institutet Ethical Committee, Stockholm, Sweden (Dnr: 2006/431-31/2), and was performed in accordance with the Declaration of Helsinki.

Vitamin D. Levels of 25OHD in serum were determined using DiaSorin immunochemical method (DiaSorin S.p.A, Saluggia, Italy) at the Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden.

Genotyping vitamin D receptor. Genomic DNA was isolated from 200 μL peripheral blood leucocytes using the DNA Blood Mini Kit (Qiagen, Hilden, Geramany). Allelic discrimination reactions were performed using TaqMan® genotyping assay (C_2404008_10; Applied Biosystems, Foster City, CA, USA) for the VDR polymorphism (rs731236 also referred to as *TaqI*). The final volume for each reaction was 15 μL consisting of 30 ng DNA and 2xTaqman Universal PCR Master Mix (Applied Biosystems). The PCR profile consisted of 95°C for 10 min. followed by 40 cycles of 92°C for 15 sec. and 60°C for 1 min. The fluorescence signal was measured with an ABI 7500 Sequence Detector (Applied Biosystems).

Statistical analysis. All statistical tests were performed using (GraphPad Software Inc, La Jolla, CA, USA) Prism v. 6.00, and $p < 0.05$ was considered statistically significant. For the comparison of age groups, the Kruskal–Wallis test was performed followed by Dunn's multiple comparisons post-test for the identification of which groups differed from one another. Unpaired, two-tailed *t*-test was used to investigate whether there were any differences between patients with and without myopathy, between women and men and between patients with and without the CC genotype. All of these data had a Gaussian distribution. The bars in the figures show mean + S.E.M. To evaluate whether 25OHD levels <50 nmol/L or the VDR polymorphism could be used as a predictor for statin-induced myopathy, Fisher's exact test was used.

Results

Baseline demography.

The study comprised 127 patients, 16 with statin-induced myopathy and 111 without myopathy. Twelve of the patients with myopathy (12/16, 75%) and 55 without myopathy (55/111, 50%) were women. The median age among patients with myopathy was 65 years (range 39–86) and among patients without myopathy 65 years (range 32–89). The patients with myopathy were all treated with a daily dose of simvastatin, 10–40 mg, average dose 25.3 mg. Of the 111 patients without myopathy, 69 were treated with a daily dose of simvastatin (10–40 mg), 33 with rosuvastatin (5–20 mg), 6 with atorvastatin (10–40 mg), two with 20 mg pravastatin and one with 10 mg fluvastatin.

25-Hydroxyvitamin D levels at baseline.

In the whole cohort, the mean level of 25OHD level was 59 ± 2 nmol/L. When patients were divided into age groups, <50, 50–59, 60–69 and >70, statistical analysis showed that the age groups >70 had significantly lower 25OHD levels compared with the other age groups ($p < 0.01$) (fig. 1). There was no significant seasonal variability in the samples throughout the year in this cohort (data not shown).

At baseline, the median 25OHD level was 50 ± 4 nmol/L among patients with myopathy and 60 ± 2 nmol/L among patients without myopathy ($p < 0.05$) (fig. 2). Nine of the myopathy patients (9/16) and 21 of the patients without myopathy (21/111) had 25OHD levels <50 nmol/L (fig. 2). Consequently, individuals with 25OHD levels <50 nmol/L before starting on statin treatment had four times higher risk of developing muscular symptoms compared with individuals having 25OHD levels >50 nmol/L (relative risk 4.2; 95% confidence interval 1.7–10.2; $p < 0.01$). There was no difference in baseline levels of 25OHD between men and women (mean values 58 ± 17 and 58 ± 18 nmol/L).

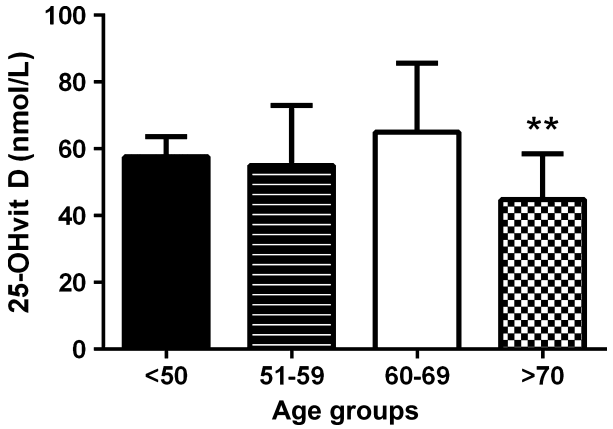

Fig. 1. 25-Hydroxyvitamin D (25OHD) levels in different age groups in the study population at baseline: <50, n = 21; 51–59, n = 30; 61–69, n = 49; >70, n = 27. The bars show mean + S.E.M. The Kruskal–Wallis test was performed followed by Dunn's multiple comparisons post-test for the identification of which groups differed from one another. In the age group >70, the patients had lower levels of 25OHD than the other age groups, $^{**}p < 0.01$.

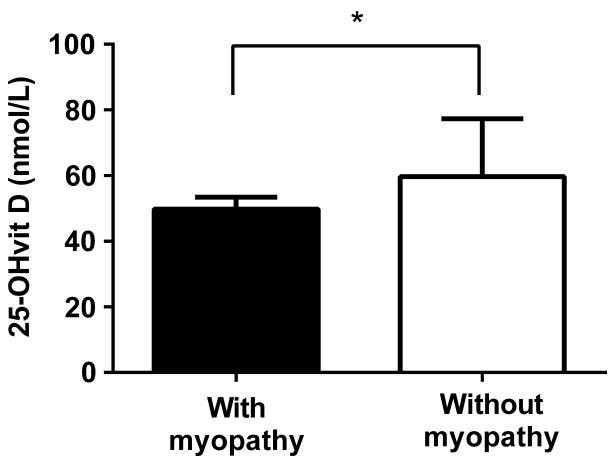

Fig. 2. Baseline levels of 25-hydroxyvitamin D (25OHD) levels in patients who developed myopathy (n = 16) and who did not (n = 111) during statin treatment. Statistical analysis showed a significant difference between the groups, $^{*}p < 0.05$. The bars show mean + S.E.M.

Vitamin D receptor polymorphism in TaqI *and the risk of statin-induced myopathy.*

DNA for genetic analysis was available for 84 of the patients, 14 with myopathy and 70 without myopathy (table 1). The observed genotype distribution in patients with and without myopathy was not different from that expected under Hardy–Weinberg equilibrium (χ^2 = 1.77E-6, $p = 0.99$). The allele frequency of VDR *TaqI* T allele in patients without myopathy was 0.64 as compared with 0.28 among the patients with myopathy. Relative to individuals with one or two T alleles (CT and TT), we found that individuals homozygous for the C allele were at significantly increased risk of developing muscular symptoms (RR 4.4; 95% CI 1.9–10.1; $p < 0.01$). There was no association between the VDR polymorphism and serum levels of 25OHD (data not shown).

Table 1.

Genotype frequencies of vitamin D receptor *TaqI* polymorphism in statin patients with myopathy and without myopathy. Individuals homozygous for the C allele were at significantly increased risk of developing muscular symptom, RR 4.4; 95% CI 1.9–10.1; $p < 0.01$, according to Fisher's exact test.

	With myopathy (%)	Without myopathy (%)
TT	1 (7)	27 (39)
TC	6 (43)	35 (50)
CC	7 (50)	8 (11)

Among patients with myopathy and the CC genotype (n = 7), the mean 25OHD concentrations were 57 ± 5 nmol/L compared to 42 ± 5 nmol/L among the other patients with myopathy and CT or TT genotype (n = 7). However, this difference did not reach statistical significance ($p = 0.07$).

Discussion

In this study, we show that patients with 25OHD levels <50 nmol/L had four times higher risk of developing statin-induced myopathy during a 1-year follow-up period. In addition, we found that individuals homozygous for a specific genetic polymorphism in VDR had more than four times increased risk of developing muscular symptoms. Our findings indicate that low 25OHD levels could be used as a marker to predict statin-induced myopathy.

The results are supportive of previous investigations. In a previous cross-sectional study of 621 statin-treated patients, the 128 patients with muscular symptoms had lower vitamin D levels than the 493 asymptomatic patients [19]. Notably, 38 of the patients with muscular symptoms were given vitamin D (50,000 units/week in 12 weeks), which resulted in recovery from muscular symptoms in 35 individuals, that is 92% [19]. In another study with a retrospective and observational design comprising 64 patients, there was a strong association between supplementation of deficient 25OHD levels and improved muscular symptoms [21].

In 150 hypercholesterolaemic patients with 25OHD levels <80 nmol/L, who were unable to tolerate statin therapy due to myopathy, vitamin D supplementation resulted in that 87% (131/150) could restart and tolerate statin therapy without muscular symptoms [20].

Two large observational studies (n = 450 and n = 5526) showed that low 25OHD levels were associated with higher incidence of statin-induced myopathy [17,18]. Recently, in a large cross-sectional study (n = 5907), it was shown that patients with 25OHD levels <40 nmol/L (15 ng/mL) had two times higher risk of suffering from statin-induced myopathy [30]. In contrast, other studies have failed to find an association between 25OHD levels and statin-induced myopathy [22–24].

It should also be noted that several studies have shown that low 25OHD levels is associated with increased risk of pain in general, not only during statin treatment [13,31,32].

This is, to our knowledge, the first study to have examined a genetic polymorphism in VDR in relation to statin-induced myopathy. The *TaqI* polymorphism is a silent SNP located in

exon 9 that is in close linkage disequilibrium with other genetic variations in the VDR gene, which is probably linked to gene regulation. Earlier reports have suggested that the C allele of this SNP is associated with increased levels of VDR [26,33,34]. However, we have recently shown that individuals homozygous for the C allele had significantly lower expression of VDR in foetal liver tissue [27]. In line with this, we here show that individuals carrying the C allele have an increased risk of statin-induced myopathy, strengthening a causal relationship between vitamin D function and myopathy. However, the genotyping data presented here should be interpreted with caution due to the limited number of individuals (n = 84) and larger studies are needed before any firm conclusions can be drawn.

The strength of the present study, compared with previously performed studies, is that the patients were thoroughly followed regarding muscular symptoms with interviews and questionnaires comprising both numeric and categorical scales. Each case was carefully evaluated in accordance with guidelines from WHO regarding adverse events to decide whether the myopathy was associated with statin treatment or not. Another advantage is that the study was carried out in Sweden with a high seasonal variability in 25OHD levels during the year due to seasonal variability in sunlight exposure. In Sweden, a significant proportion of the population has low or deficient 25OHD levels during the winter season [35,36].

A weakness of this study is the observational design, which inferred that we had no influence on the different types of statins or doses used. Thus, by including all types of statins and many different doses, a significant heterogeneity is present. However, this is also a strength of the study as the patients represent an unselected cohort in primary care in Sweden, which is in contrast to randomized and placebo-controlled trials with carefully selected patients following strict protocols of dose titration.

It could be argued that an association between low 25OHD levels and statin-induced myopathy could be explained by an age factor; that is, that older people have lower 25OHD levels and are at increased risk of developing myopathy. However, the median age was the same between the patients with and without myopathy.

In conclusion, in this study, we could confirm results from previous studies that low 25OHD levels are associated with increased risk of statin-induced myopathy. Our results indicate that 25OHD levels <50 nmol/L might be a useful marker to predict statin-induced myopathy and could be measured before starting statin therapy. In addition, the finding that the VDR polymorphism *TaqI* was associated with myopathy suggests a role for vitamin D in myopathy.

Randomized and placebo-controlled trials testing the hypothesis that vitamin D supplementation could decrease statin-induced myopathy are warranted.

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Conflict of Interest

The authors have no conflict of interest to declare.

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