RESEARCH ARTICLE

ApaI polymorphism of vitamin D receptor affects health-related quality of life in patients with primary sclerosing cholangitis

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Abstract

Background

Polymorphisms of vitamin D receptor (VDR) contribute to the pathogenesis of multiple autoimmune conditions.

Methods

We investigated the incidence of VDR polymorphisms (rs1544410-BsmI; rs7975232-ApaI; rs731236-TaqI) in a group of patients with primary sclerosing cholangitis (PSC, n = 275) and in healthy controls (n = 376). Additionally, correlations of the VDR polymorphisms with clinical and biochemical factors of the disease were analysed.

Results

The genotype and allele distributions of these polymorphisms in PSC patients were similar to those observed in controls. However, the ApaI polymorphism was associated with an impaired health-related quality of life (HRQoL). The generic SF-36 questionnaire showed that the Role-Physical (p = 0.01), Role-Emotional (p = 0.01), Physical Component Summary (p = 0.01) and Mental Component Summary (p = 0.003) scores were significantly affected. Similarly, the disease-specific questionnaires, PBC-40 and PBC-27, demonstrated that carriers of the C allele suffered from more severe Itch (p = 0.03 assessed by PBC-40 and PBC-27), more Fatigue (p = 0.02 assessed by PBC-40 and PBC-27) and Impaired Cognitive Capacity (p = 0.04 and p = 0.03). Correspondingly, individuals who were AA homozygotes (non-carriers of the C allele of ApaI) had higher summary scores for the Physical (p = 0.01) and Mental Components (p = 0.006) measured with SF-36. Moreover, they experienced less Itch (p = 0.03) and less Fatigue (p = 0.03) and had better Cognitive Abilities (p = 0.04) as assessed by the PBC-40 and PBC-27 questionnaires. No associations between other VDR polymorphisms and clinical or laboratory findings were made.
Conclusion
In summary, this study is the first to show that the Apal polymorphisms in VDR may exert an effect on disease-related symptoms and quality of life in patients with PSC.

Introduction
Primary sclerosing cholangitis (PSC), which frequently co-exists with inflammatory bowel disease, is a chronic cholestatic liver condition that affects both the small and large bile ducts. It occurs predominantly in males and often remains asymptomatic in the early stages of the disease. Nevertheless, progressing biliary tree damage ultimately leads to chronic cholestasis, recurrent cholangitis and liver cirrhosis in a high proportion of affected individuals [1], impairing the health-related quality of life (HRQoL)[2–4]. Moreover, patients with PSC are at an increased risk of cholangiocarcinoma, a primary biliary cancer with a fatal prognosis [5]. The effectiveness of pharmacological treatment remains controversial and insufficient [1,6], and liver transplantation remains the only curative option. Further recognition of its pathologic mechanisms may help to identify potential effective therapeutic targets. Unfortunately, the pathogenesis of PSC remains incompletely understood and is most likely related to the multimodal influences of inflammatory, autoimmune, genetic and infective factors[7]. Presumably, in genetically susceptible subjects, environmental factors trigger a pathological immune response that ultimately leads to lymphocyte migration, inflammation, and fibrotic damage of the biliary tree. While aiming to further understand PSC pathogenesis, several studies have focused on immunopathogenetic mechanisms, but the links between immunity and PSC remain unsatisfactorily explained.

1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃) exerts multiple immunomodulatory actions, and beyond its crucial role in mineral homeostasis, it is now believed to represent an important component of the immune response[8]. Strong evidence has shown that a disturbance in 1,25(OH)₂D₃ metabolism plays a role in the pathogenesis of several autoimmune diseases [9–15], including autoimmune liver disorders [16,17]. The effects of 1,25(OH)₂D₃ on target genes are mediated by a ligand-activated nuclear receptor, the vitamin D receptor (VDR) [18]. Several polymorphisms in the VDR gene have been described, but their effects on VDR function are poorly understood. Three of them—rs1544410 (BsmI), rs7975232 (ApaI), and rs731236 (TaqI)—have been linked to other chronic cholestatic conditions, including primary biliary cholangitis (PBC) [16,17,19–23], and our recent study has highlighted the association between the BsmI and TaqI variants and the disease severity [24].

In the study, we investigated the prevalence of VDR polymorphisms in a homogenous cohort of well-characterized Polish patients with PSC. Additionally, associations between VDR receptor polymorphisms were analysed in the context of health-related quality of life along with clinical as well as laboratory features of the disease.

Materials and methods
Patients
Two hundred and seventy-five patients (182 males, 93 females; median age at diagnosis 55 years, range 28–90 years) with PSC were recruited in two medical centres (Pomeranian Medical University, Szczecin, Poland and Medical University of Warsaw, Warsaw, Poland) between 2006 and 2015. The diagnosis of PSC was based on the MRCP/ERCP findings, per the EASL
recommendations[25]. IgG4 cholangitis was excluded based on the laboratory and clinical profile. The demographic characteristic and main laboratory data of included subjects are presented in Table 1.

A cohort of 376 (age range 18–66 years) blood donors from the Regional Blood Donor Centre in Szczecin (Poland) was investigated. All subjects had a medical check-up, and a good state of health was a prerequisite to qualify for blood donation. Each participant provided his/her written informed consent. All consent records are deposited either in the Liver and Internal Medicine Unit, MUW, or in the Department of Medical Biology, PMU. The study protocol and consent procedure conform to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and were approved by the Ethics Committee of Pomeranian Medical University.

**VDR genotyping**

DNA from peripheral blood mononuclear cells was isolated using the DNeasy Blood & Tissue Kit (Qiagen). Oligonucleotide primers and TaqMan probes for VDR polymorphisms (rs7975232, rs15444410, rs731236) were designed and synthesized by Applied Biosystems (Assay ID: C_28977635_10, C_8716062_10, C_2404008_10, resp.). The fluorescence data were analysed with allelic discrimination 7500 Software v.2.0.2.

In addition to a nucleotide code, the description of the VDR genotype in the tables includes letters enclosed in square brackets that represent previously described nomenclature derived from a restriction-fragment length polymorphism (RFLP) analysis. The presence and absence of a restriction site are denoted with a lowercase and uppercase letter, respectively ([b, B] for BsmI; [a, A] for ApaI; [t, T] for TaqI) that also refers to a specific base change.

**HRQoL assessment**

HRQoL is a multidimensional parameter that comprehensively assesses various aspects of human well-being, such as physical and cognitive capabilities, emotional status, and psychosocial adjustment, in the context of health and disease. HRQoL can be measured by generic or disease-specific questionnaires. In our study, we used one generic (Medical Outcome Study Short Form-36, SF-36) and two disease-specific (PBC-40 and PBC-27) tools. The SF-36 was designed in 1992 to measure the HRQoL in various populations and a wide variety of medical conditions.

Table 1. Demographic data of analysed subjects.

| Feature                  | PSC (n = 275) | Control group (n = 376) |
|--------------------------|--------------|------------------------|
| Age (median; range)      | 55 (28–90)   | 27.8 (18–66)           |
| Gender (M/F)             | 182/93       | 344/32                 |
| Haemoglobin (median; range) IU/l | 13.2 (6.6–53.9) | N/A                    |
| AST (median; range) IU/l | 92 (17–1628) | N/A                    |
| ALT (median; range) IU/l | 130 (16–1411) | N/A                    |
| ALP (median; range), IU/l| 354 (33–2061) | N/A                    |
| GGT (median; range), IU/l| 332.5 (24–3102) | N/A                    |
| Bilirubin (median; range), mg/dl | 1.3 (0.2–27)   | N/A                    |
| Cholesterol (median; range), mg/dl | 209 (72–871)  | N/A                    |
| Triglycerides (median; range), mg/dl | 79 (29–489)     | N/A                    |

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline Phosphatase; GGT: Gamma-glutamyl transferase; N/A—Not Applicable

https://doi.org/10.1371/journal.pone.0176264.t001
conditions to allow the possibility of comparing several health states\cite{26}. The SF-36 includes 36 items divided into eight domains of physical health (Physical Functioning, Role Limitation-Physical, Bodily Pain and General Health) and mental health (Vitality, Social Functioning, Role Limitation-Emotional and Mental Health). Two summary scores, Physical Component and Mental Component, can also be calculated. Scale scores range between 0 (denoting the most impaired HQoL) and 100 (ideal well-being). License No. QM011392-QualityMetric CT133208/OP018661 was obtained for the use of the SF-36 questionnaire in this study.

The PBC-40 questionnaire and its simplified form, PBC-27, were designed for the assessment of disease-specific symptoms among patients with PBC \cite{27,28}. Both questionnaires were recently validated by our group for use in PSC \cite{2}. PBC-40 consists of 40 questions in 5 domains including Cognition, Itch, Fatigue, Social-Emotional and Other Symptoms that are assessed using a five-point scale (1 = never to 5 = always), with higher scores denoting a greater symptom impact and poorer HRQoL. The possible range of each domain is as follows: Symptom domain (7–35), Itch (3–15), Fatigue (11–55), Cognitive (6–30), Social and Emotional (13–65) points. In the PBC-27 questionnaire, 27 items are grouped into 7 domains as follows: Other Symptoms (possible range: 3–15 points), Dryness (2–10 points), Itch (3–15 points), Fatigue (8–40 points), Cognitive (5–25 points), Emotional (3–15 points) and Social (3–15 points), with the same 5-point scale of evaluation.

Statistics

All statistical analyses were performed using the Stat-View-5 Software (SAS Institute, Cary, NC, US). The genotype and allele frequencies were compared between patients and controls using Fisher’s exact probability test. The odds ratio (OR) and 95% confidence interval (CI) for each variable were also estimated. The analysis of genotype frequency in regards to the clinical characteristics and HRQoL assessment of PSC patients was performed using ANOVA with Fisher’s protected least significant difference (Fisher’s. PLSD). Data were evaluated as the mean ± standard deviation (SD) for continuous variables. A two-sided significance level of 0.05 was considered to indicate a statistically significant difference.

Results

No significant differences in the genotype or allelic frequencies of the VDR polymorphisms between the PBC patients and healthy controls were seen (Tables 2 and 3). However, one of the examined VDR polymorphisms, the Apal variant, showed a negative effect on the patients’ well-being as measured with the generic and disease-specific questionnaires.

Data for HRQoL are available for 167 patients. Significant negative associations between the C [a] allele of rs7975232 and 4 domains of SF-36 were found. These included the Role-Physical (\(P = 0.01\)), Role-Emotional (\(P = 0.01\)), Physical Component Summary (\(P = 0.01\)), and Mental Component Summary (\(P = 0.003\)) scores. Similarly, the disease-specific questionnaires, including PBC-40 and PBC-27, demonstrated that subjects who were C [a] carriers of rs7975232 suffered from more severe Itch (\(P = 0.03\) and \(P = 0.03\)) and Fatigue (\(P = 0.02\) and \(P = 0.02\)) and an Impaired Cognitive Capacity (\(P = 0.04\) and \(P = 0.03\)), respectively. These data are summarized in Table 4.

Correspondingly, the AA homozygotes of the rs7975232 who did not have the C [a] allele had significantly higher Physical (\(P = 0.01\) vs CC, and \(P = 0.04\) vs AC) and Mental Component Summary scores as measured with SF-36 (\(P = 0.006\) vs CC, and \(P = 0.009\) vs AC), respectively (Table 5). No correlations were found between the genotypes and allelic analyses of rs1544410 (BsmI) or rs731236 (TaqI) and the quality of life features using the SF-36, PBC-40 and PBC-27 questionnaires (Table 4).
The presence of these polymorphisms did not correlate with analysed clinical features such as gender, age and cirrhosis at presentation or liver biochemistry at diagnosis (S1 Table).

Discussion

In this study, we have analysed the prevalence of three common VDR polymorphisms (Apal-rs7975232, BsmI-rs1544410, TaqI-rs731236) and investigated their potential relationships with the severity of disease-related symptoms in a well characterized cohort of Polish patients with PSC. Despite similar distributions of the VDR variants in patients with PSC and in healthy subjects, our study clearly indicated that the VDR polymorphisms impact the clinical phenotype of PSC patients. We have shown that the Apal variant of the VDR gene profoundly impairs well-being among patients with PSC as measured with the general and disease-specific questionnaires. Apal allele a was associated with a worse HRQoL as measured by generic SF-36 in the following domains: Role Limitation-Physical, Role Limitation-Emotional and the Physical and Mental Component Summaries. Moreover, the analysis of the PBC-40/PBC-27 domains showed that HRQoL scores for the carriers of Apal allele a were almost all impaired; the impaired scores included Itch, Fatigue and Cognitive in both questionnaires and the Social and Emotional domain in PBC-40. We obtained similar results when analysing the genotype

Table 2. Genotype counts for VDR polymorphisms (rs1544410, rs7975232, rs731236) in PSC patients and in controls.

| Genotype | PSC (%) (n = 275) | Controls (%) (n = 376) | P* Value | X² | OR (95% CI) |
|----------|-------------------|------------------------|-----------|----|-------------|
| rs1544410 (BsmI) | | | | | |
| AA [BB] | 40 (14.5%) | 44 (11.7%) | 0.3 | 1.14 | 1.28 (0.8–2.0) |
| GA [bB] | 121 (44.0%) | 160 (42.6%) | 0.7 | 0.1 | 1.06 (0.8–1.5) |
| GG [bb] | 114 (41.5%) | 172 (45.7%) | 0.3 | 1.2 | 0.8 (0.6–1.2) |
| rs7975232 (Apal) | | | | | |
| AA [AA] | 67 (24.4%) | 74 (19.7%) | 0.2 | 2.1 | 1.3 (0.9–1.9) |
| AC [aA] | 124 (45.1%) | 196 (52.1%) | 0.8 | 3.1 | 0.7 (0.6–1.03) |
| CC [aa] | 84 (30.5%) | 106 (28.2%) | 0.5 | 0.4 | 1.1 (0.8–1.6) |
| rs731236 (TaqI) | | | | | |
| TT [TT] | 116 (42.2%) | 172 (45.7%) | 0.5 | 0.6 | 0.9 (0.6–1.2) |
| TC [Tt] | 124 (45.1%) | 160 (42.6%) | 0.5 | 0.4 | 1.1 (0.8–1.5) |
| CC [tt] | 35 (12.7%) | 44 (11.7%) | 0.7 | 0.2 | 1.1 (0.7–1.8) |

* Fisher’s exact probability test; Chi-squared test for categorical variables

PSC: Primary Sclerosing Cholangitis; OR: odds ratio; CI: confidence interval.

https://doi.org/10.1371/journal.pone.0176264.t002

Table 3. Allele association for VDR in patients with PSC and control subjects.

| SNP | Allele | PSC (%) (n = 275) | Controls (%) (n = 376) | P* Value | X² | OR (95% CI) |
|-----|--------|-------------------|------------------------|-----------|----|-------------|
| rs1544410 (BsmI) | A/G [Bb] | 201 (36.5%)/349 (63.5%) | 248 (33%)/504 (67%) | 0.2 | 1.8 | 1.2 (0.9–1.4) |
| rs7975232 (Apal) | A/C [Aa] | 258 (46.9%)/292 (53.1%) | 344 (45.7%)/408 (44.3%) | 0.6 | 6.9 | 1.1 (0.8–1.3) |
| rs731236 (TaqI) | C/T [t/T] | 194 (35.3%)/356 (64.7%) | 248 (33%)/504 (67%) | 0.4 | 0.7 | 1.1 (0.9–1.4) |

* Fisher’s exact probability test;
PSC: Primary Sclerosing Cholangitis; OR: odds ratio; CI: confidence interval.

https://doi.org/10.1371/journal.pone.0176264.t003
Table 4. Allelic analysis of rs1544410, rs7975232 and rs731236 in relation to SF-36, PBC-40 and PBC-27 domains.

| SF-36          | rs1544410 (BsmI) | rs7975232 (Apal) | rs731236 (TaqI) |
|---------------|------------------|------------------|-----------------|
| Physical      |                  |                  |                 |
| functioning   | A [B]            | G [b]            | P*              | A [A] | C [a] | P*  | [T] | [t] | P*  |
| Role-Physical | 85.2±4.2         | 81.2±1.9         | NS              | 87.7±3.1 | 80.7±2.1 | NS | 79.0±2.9 | 85.2±2.2 | NS |
| Bodily Pain   | 73.0±7.2         | 61.8±3.5         | NS              | 77.0±5.7 | 59.4±3.7 | 0.01 | 57.2±5.0 | 68.8±4.0 | NS |
| General Health| 73.4±5.6         | 72.9±2.5         | NS              | 80.2±4.2 | 70.7±2.6 | NS | 70.5±3.7 | 74.9±2.9 | NS |
| Vitality      | 50.3±3.8         | 48.0±1.8         | NS              | 53.5±4.0 | 46.7±1.9 | NS | 46.8±2.5 | 49.6±2.5 | NS |
| Social        | 68.7±5.2         | 64.6±2.0         | NS              | 72.1±3.9 | 63.1±2.2 | NS | 61.8±2.9 | 68.0±2.6 | NS |
| Role-Emotional| 83.5±6.9         | 72.3±3.2         | NS              | 87.1±5.1 | 69.8±3.1 | 0.01 | 67.8±4.4 | 78.9±3.8 | NS |
| Mental Health | 68.6±3.8         | 64.9±1.7         | NS              | 69.3±3.1 | 64.3±1.7 | NS | 62.2±2.4 | 68.2±1.9 | NS |
| Physical      | 67.9±4.6         | 64.9±1.8         | NS              | 73.2±3.5 | 62.9±1.9 | 0.01 | 62.6±2.6 | 67.5±2.3 | NS |
| Component     | 65.9±4.3         | 61.5±1.8         | NS              | 71.3±3.3 | 59.3±1.9 | 0.003 | 58.6±2.5 | 65.0±2.2 | NS |

PBC-40

| Other Symptom | A [B]            | G [b]            | P*              | A [A] | C [a] | P*  | [T] | [t] | P*  |
|---------------|------------------|------------------|-----------------|-------|-------|-----|-----|-----|-----|
| Itch          | 13.7±0.8         | 12.6±0.4         | NS              | 12.5±0.7 | 12.9±0.4 | NS | 12.8±0.5 | 12.7±0.5 | NS |
| Fatigue       | 3.8±0.6          | 4.7±0.3          | NS              | 3.5±0.5 | 5.0±0.4 | 0.03 | 5.1±0.5 | 4.3±0.4 | NS |
| Cognitive     | 22.0±1.6         | 24.5±0.8         | NS              | 21.1±1.3 | 25.1±0.9 | 0.02 | 24.8±1.1 | 23.5±1.0 | NS |
| Social and    | 10.1±0.8         | 10.9±0.4         | NS              | 9.3±0.6 | 11.2±0.4 | 0.04 | 10.9±0.5 | 10.5±0.5 | NS |
| Emotional     | 27.0±2.1         | 29.8±0.8         | NS              | 26.4±1.5 | 30.3±0.9 | 0.03 | 30.7±1.1 | 28.2±1.0 | NS |

PBC-27

| Other Symptom | A [B]            | G [b]            | P*              | A [A] | C [a] | P*  | [T] | [t] | P*  |
|---------------|------------------|------------------|-----------------|-------|-------|-----|-----|-----|-----|
| Dryness       | 6.4±0.5          | 6.2±0.2          | NS              | 6.0±0.4 | 6.3±0.2 | NS | 6.4±0.3 | 6.1±0.2 | NS |
| Itch          | 3.9±0.3          | 3.9±0.1          | NS              | 3.6±0.2 | 4.1±0.1 | NS | 4.1±0.2 | 3.9±0.1 | NS |
| Fatigue       | 3.8±0.6          | 4.7±0.3          | NS              | 3.4±0.5 | 5.0±0.3 | 0.03 | 5.0±0.5 | 4.2±0.3 | NS |
| Cognitive     | 16.6±1.1         | 18.6±0.6         | NS              | 16.9±0.9 | 19.0±0.6 | 0.02 | 19.1±0.8 | 17.6±0.7 | NS |
| Emotional     | 6.4±0.5          | 6.7±0.2          | NS              | 6.0±0.4 | 6.8±0.3 | NS | 6.8±0.3 | 6.6±0.3 | NS |
| Social        | 6.2±0.7          | 6.9±0.2          | NS              | 5.9±0.5 | 7.1±0.3 | NS | 7.2±0.3 | 6.5±0.4 | NS |

* ANOVA with Fisher’s protected least significant difference (PLSD); NS: not significant.

https://doi.org/10.1371/journal.pone.0176264.t004

Profiles; the heterozygotes and homozygotes carrying Apal allele a showed impaired well-being scores in the aforementioned domains of SF-36 and PBC-40/PBC-27.

Impaired quality of life is often associated with symptoms such as chronic fatigue and is quite frequently seen in patients with chronic cholestasis [29]. Few reports have already indicated the negative impact of PSC on HRQoL [3,4]. In our previous study, we observed an impairment in quality of life for patients with PSC compared to healthy individuals, and our data highlighted a significant impact of female gender in predicting worse quality of life [2]. Our current project increases our knowledge on the impact of genetic variations in PSC on patients’ well-being, as this is the first study to focus on HRQoL assessment in this context.

Our results suggest that although the analysed VDR variants do not increase the susceptibility to PSC, they may have an impact on the severity of disease-related symptoms. The mechanistic background of this association remains difficult to explain because the functional effects of VDR polymorphisms are still poorly understood. Because the location of Apal polymorphism is intronic, it might affect alternative splicing of the VDR mRNA or be relevant as an enhancer that augments the transcription of an associated gene. It is also possible that Apal may be a

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Table 5. Relationship between the rs7975232 VDR polymorphisms and features of the SF-36, PBC-40 and PBC-27 questionnaires.

|               | rs7975232 (Apal) |          |          |          |          |          |
|---------------|------------------|----------|----------|----------|----------|----------|
|               | AA [AA]         | AC [Aa]  | CC [aa]  | P* AA vs AC | P* AA vs CC | P* AC vs CC |
| **SF-36**     |                  |          |          |          |          |          |
| Physical      | 87.7±3.6        | 81.1±2.7 | 80.2±3.5 | NS        | NS        | NS        |
| Functioning   | 77.0±5.8        | 56.0±4.9 | 64.3±5.7 | **0.01**  | NS        | NS        |
| Role-Physical | 80.2±4.2        | 69.3±3.5 | 72.7±4.1 | **0.06**  | NS        | NS        |
| Bodily Pain   | 53.6±4.0        | 47.6±2.5 | 45.4±3.3 | NS        | NS        | NS        |
| General Health| 58.7±3.5        | 52.9±2.1 | 54.0±3.0 | NS        | NS        | NS        |
| Vitality      | 72.1±3.9        | 62.7±3.0 | 63.6±3.4 | 0.06      | NS        | NS        |
| Social        | 87.1±5.1        | 71.3±4.5 | 67.7±5.0 | **0.03**  | **0.01**  | NS        |
| Functioning   | 69.3±3.1        | 66.1±2.2 | 61.7±3.0 | NS        | 0.07      | NS        |
| Mental Health | 73.2±3.5        | 62.2±2.5 | 63.9±3.1 | **0.01**  | 0.04      | NS        |
| Physical      | 71.3±3.3        | 59.5±2.4 | 59.3±3.1 | **0.006** | **0.009** | NS        |
| Component     |                 |          |          |          |          |          |
| Summary       | PBC-40           |          |          |          |          |          |
| Other Symptom | 12.5±0.7        | 12.8±0.5 | 13.0±0.7 | NS        | NS        | NS        |
| Itch          | 3.4±0.5         | 5.1±0.5  | 4.8±0.6  | **0.03**  | NS        | NS        |
| Fatigue       | 21.1±1.2        | 25.3±1.1 | 24.8±1.5 | **0.03**  | 0.07      | NS        |
| Cognitive     | 9.3±0.6         | 11.2±0.6 | 11.1±0.7 | **0.04**  | 0.07      | NS        |
| Social and    | 26.3±1.5        | 30.4±1.2 | 30.2±1.4 | **0.04**  | 0.08      | NS        |
| Emotional     |                 |          |          |          |          |          |
| Social        | 6.0±0.4         | 6.3±0.3  | 6.3±0.4  | NS        | NS        | NS        |
| Dryness       | 3.6±0.2         | 4.0±0.2  | 4.2±0.3  | NS        | NS        | NS        |
| Itch          | 3.5±0.5         | 5.1±0.5  | 4.8±0.6  | **0.03**  | NS        | NS        |
| Fatigue       | 16.0±0.9        | 18.9±0.8 | 19.1±1.1 | **0.04**  | **0.03**  | NS        |
| Cognitive     | 7.7±0.5         | 9.4±0.5  | 9.3±0.6  | **0.04**  | 0.07      | NS        |
| Emotional     | 6.0±0.4         | 7.0±0.3  | 6.7±0.4  | NS        | NS        | NS        |
| Social        | 6.0±0.5         | 7.3±0.4  | 6.9±0.4  | **0.05**  | NS        | NS        |

*Fisher's. PLSD; NS: not significant.

https://doi.org/10.1371/journal.pone.0176264.t005

Interestingly, the Apal polymorphism was associated with impaired cognitive function in elderly Chinese subjects [31] and with cognitive impairment and depression in elderly Dutch patients [32]. Another functional polymorphism of VDR, namely, Fokl, was found to be associated with cognitive decline in American patients with Parkinson’s disease [33]. Moreover, correction of vitamin D deficiency exerted an ameliorating effect on chronic fatigue in a large cohort of more than 170 subjects presenting with this symptom to their general practitioners [34], and Vitamin D replacement significantly improved depressive symptoms in women with chronic liver diseases [35].

Although our study does not fully elucidate the mechanism that underlies the observed association, our data may be of clinical relevance. In the liver, VDR is expressed in non-parenchymal cells and biliary epithelial cells [36]. After binding its ligand, VDR forms a heterodimer with the retinoid X receptor (RXR) to modulate divergent pathways ranging from calcium metabolism to immune system homeostasis. Furthermore, 1,25(OH)2D3, lithocholic acid and its metabolites have been shown to act as VDR ligands [37]. Moreover, VDR-related pathways are engaged in the regulation of bile acid synthesis and detoxification [38,39]. These findings,
and especially the data that show how common 1,25(OH)$_2$D$_3$ deficiency is in autoimmune conditions, suggest that dysfunction of VDR may play a potential role in cholestatic liver injury. Thus, several studies have been performed regarding homeostasis of vitamin D in chronic cholestasis, but the vast majority have focused on PBC, while little has been done in relation to PSC. PBC genetic studies of the VDR have repeatedly indicated the association of the BsmI polymorphism with susceptibility to PBC[17,20–22,40]. Moreover, our recent study indicated the relationship between the BsmI and TaqI polymorphisms of the VDR gene and the presence of liver cirrhosis and advanced fibrosis[24].

The data regarding vitamin D-VDR signalling in PSC are more scarce. Most available analyses concerning PSC specifically focus on the serum levels of 1,25(OH)$_2$D$_3$. To date, there are no available studies in the setting of the VDR gene variability in PSC. Our study is the first analysis of three polymorphisms that have been previously indicated as risk factors of PBC and other autoimmune conditions. We showed that there is no relationship between VDR variants and susceptibility to PSC. These findings are in accordance with previous genome-wide association studies (GWAS), which recognize the strongest genetic risk for PSC within the major histocompatibility complex (MHC) and within several other loci that contain genes that regulate immune self-recognition and adaptive immunity, but not within the VDR gene[41].

Two decades ago, Jorgensen R.A. et al. found vitamin D deficiency among patients with PSC, particularly in patients with advanced disease who were evaluated for liver transplantation. In the pretransplantation group, lower levels of 1,25(OH)$_2$D$_3$ were observed in over half of patients, compared to 14% of subjects in the less advanced clinical condition[42]. Further studies have shown that vitamin D deficiency is commonly seen in patients with chronic liver disease regardless of the underlying aetiology of the liver injury and that it correlates with fibrosis progression[43–45]. Moreover, vitamin D deficiency has been proven to impair the course of liver disease and prognosis [46–48]. The evidence deriving from in vitro and animal studies suggest that supplementation of vitamin D may exert beneficial effects in PSC. A study by Hochrath et al. demonstrated that vitamin D diminishes hepatic inflammation in Abcb4−/− mice, a reproducible animal model of sclerosing cholangitis[49]. Moreover, vitamin D inhibits activation and proliferation of murine hepatic stellate cells, which produce the extracellular matrix proteins that are deposited in liver fibrosis. These studies suggest that 1,25(OH)$_2$D$_3$ is potentially an attractive therapeutic agent that may ameliorate cholestatic liver injury. In view of these and our findings, further studies should focus on the potential influence of vitamin D on laboratory parameters as well as disease-related symptoms.

The fact that we did not measure the serum concentration of 1,25-dihydroxyvitamin D can be considered a limitation of our data. However, our recent study clearly demonstrated a significant reduction in Vitamin D receptor mRNA and protein expression in liver tissues from patients with PSC [50]. This phenomenon may clearly decrease the hepatic availability of Vitamin D followed by a limitation to its cellular effects.

Conclusions

In conclusion, our study is the first to address the relationship between polymorphisms within the VDR gene and the clinical characteristics of PSC. We observed a profound effect by the Apal variants on disease-related symptoms in the studied cohort. The explanation of these findings is hindered by the unknown functional effects of VDR gene variations. Further studies are needed to investigate the pathophysiological background of the observed association and to check if the modulation of vitamin D-VDR signalling exerts beneficial effects on the clinical course of the disease.
Supporting information

S1 Table. Clinical and laboratory data depending on analyzed polymorphisms. (DOC)

Author Contributions

Conceptualization: AK-P MM.
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Investigation: AK-P EW.
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Resources: DJ EW.
Software: AKP EW.
Supervision: MM PM.
Validation: AK-P.
Visualization: AK-P.
Writing – original draft: AK-P.
Writing – review & editing: AK_P EW MM PM.

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