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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 *ApaI* 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 D_3 ($1,25(OH)_2D_3$) 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 $1,25(OH)_2D_3$ metabolism plays a role in the pathogenesis of several autoimmune diseases [9–15], including autoimmune liver disorders [16,17]. The effects of $1,25(OH)_2D_3$ 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

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*

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conditions to allow the possibility of comparing several health states[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 [27,28]. Both questionnaires were recently validated by our group for use in PSC [2]. PBC-40 consists 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).

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*	X^2	*OR (95% CI)*
rs1544410 (*Bsml*)					
AA [*BB*]	40 (14.5%)	44 (11.7%)	*0.3*	1.14	1.28 (0.8–2.0)
GA [*bB*]	121 (44.0%)	160 (42.6.2%)	*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 (*Taql*)					
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.

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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, *Bsml*-rs15444410, *Taql*-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 3. Allele association for *VDR* in patients with PSC and control subjects.

SNP	Allele	PSC (n = 275)	Controls (n = 376)	*P* Value*	X^2	OR (95%CI)
rs1544410 (*Bsml*)	A/G [*B/b*]	201 (36.5%)/349 (63.5%)	248 (33%)/504 (67%)	*0.2*	1.8	1.2 (0.9–1.4)
rs7975232 (*Apal*)	A/C [*A/a*]	258 (46.9%)/292 (53.1%)	344 (45.7%)/408 (44.3%)	*0.6*	6.9	1.1 (0.8–1.3)
rs731236 (*Taql*)	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.

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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 (*ApaI*)			rs731236 (*TaqI*)		
	A [B]	***G [b]***	***P****	***A [A]***	***C [a]***	***P****	***[T]***	***[t]***	***P****
Physical functioning	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*
Role-Physical	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*
Bodily Pain	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*
General Health	50.3±5.8	48.0±1.8	*NS*	53.5±4.0	46.7±1.9	*NS*	46.8±2.5	49.6±2.5	*NS*
Vitality	54.7±4.6	54.6±1.7	*NS*	58.7±3.5	53.4±1.7	*NS*	53.1±2.4	55.9±2.1	*NS*
Social Functioning	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 Component Summary	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*
Mental Component Summary	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	**rs1544410 (*BsmI*)**			**rs7975232 (*ApaI*)**			**rs731236 (*TaqI*)**		
	A [B]	***G [b]***	***P****	***A [A]***	***C [a]***	***P****	***[T]***	***[t]***	***P****
Other Symptom	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*
Itch	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*
Fatigue	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*
Cognitive	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*
Social and 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	**rs1544410 (*BsmI*)**			**rs7975232 (*ApaI*)**			**rs731236 (*TaqI*)**		
	A [B]	***G [b]***	***P****	***A [A]***	***C [a]***	***P****	***[T]***	***[t]***	***P****
Other Symptom	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*
Dryness	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*
Itch	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*
Fatigue	16.6±1.1	18.6±0.6	*NS*	16.0±0.9	19.0±0.6	***0.02***	19.1±0.8	17.6±0.7	*NS*
Cognitive	8.3±0.6	9.1±0.4	*NS*	7.7±0.5	9.4±0.4	***0.03***	9.2±0.5	8.8±0.4	*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.

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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 the 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

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

SF-36	rs7975232 (*ApaI*)					
	AA [*AA*]	AC [*Aa*]	CC [*aa*]	*P** AA vs AC	*P** AA vs CC	*P** AC vs CC
Physical Functioning	87.7±3.6	81.1±2.7	80.2±3.5	*NS*	*NS*	*NS*
Role-Physical	77.0±5.8	56.0±4.9	64.3±5.7	***0.01***	*NS*	*NS*
Bodily Pain	80.2±4.2	69.3±3.5	72.7±4.1	*0.06*	*NS*	*NS*
General Health	53.6±4.0	47.6±2.5	45.4±3.3	*NS*	*NS*	*NS*
Vitality	58.7±3.5	52.9±2.1	54.0±3.0	*NS*	*NS*	*NS*
Social Functioning	72.1±3.9	62.7±3.0	63.6±3.4	*0.06*	*NS*	*NS*
Role-Emotional	87.1±5.1	71.3±4.5	67.7±5.0	***0.03***	***0.01***	*NS*
Mental Health	69.3±3.1	66.1±2.2	61.7±3.0	*NS*	*0.07*	*NS*
Physical Component Summary	73.2±3.5	62.2±2.5	63.9±3.1	***0.01***	***0.04***	*NS*
Mental Component Summary	71.3±3.3	59.5±2.4	59.3±3.1	***0.006***	***0.009***	*NS*
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±06	11.1±07	***0.04***	*0.07*	*NS*
Social and Emotional	26.3±1.5	30.4±1.2	30.2±1.4	***0.04***	*0.08*	*NS*
PBC-27						
Other Symptom	6.0±04	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.

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genetic marker for other truly functional variations elsewhere in the *VDR* gene that are in linkage disequilibrium with the identified polymorphism[30].

Interestingly, the *ApaI* 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, *FokI*, 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 homoeostasis. Furthermore, $1,25(OH)_2D_3$, 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$. 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 *ApaI* 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 date depending an analyzed polymorphisms.
(DOC)

Author Contributions

Conceptualization: AK-P MM.

Data curation: AK-P EW.

Formal analysis: AK-P EW.

Funding acquisition: PM.

Investigation: AK-P EW.

Methodology: AK-P MM.

Project administration: AK-P.

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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