Genetic associations of vitamin D receptor polymorphisms with advanced liver fibrosis and response to pegylated interferon-based therapy in chronic hepatitis C

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Vitamin D receptor (VDR) modulates host immune responses, including interferon signaling. This study aimed to investigate the associations of *VDR* polymorphisms with advanced liver fibrosis and response to pegylated interferon (PEG-IFN)-based therapy in patients with chronic hepatitis C virus (HCV) infection. In total, 554 Thai patients with chronic HCV infection treated with a PEG-IFN-based regimen were enrolled. Six single-nucleotide polymorphisms (SNPs) were genotyped: the *IL28B* C>T (rs12979860) SNP and five *VDR* SNPs, comprising *FokI* T>C (rs2228570), *BsmI* C>T (rs1544410), *Tru9I* G>A (rs757343), *ApaI* C>A (rs7975232), and *TaqI* A>G (rs731236). In total, 334 patients (60.3%) achieved sustained virological response (SVR), and 255 patients (46%) were infected with HCV genotype 1. The bAt (CCA) haplotype, consisting of the *BsmI* rs1544410 C, *ApaI* rs7975232 C, and *TaqI* rs731236 A alleles, was associated with poor response to PEG-IFN-based therapy. The *IL28B* rs12979860 CT/TT genotypes (OR=3.66, 95%CI: 2.26-5.91, p<0.001), bAt haplotype (OR=2.12, 95%CI: 1.11-4.05, p=0.02), pre-treatment serum HCV RNA (logIU/ml; OR=1.75, 95%CI: 1.33-2.31, p<0.001), and advanced liver fibrosis (OR=1.60, 95%CI: 1.05-2.44, p=0.03) independently predicted poor response. Patients with the bAt haplotype were more likely to have poor response compared to patients with other haplotypes (41.4% vs 21.9%, p=0.03). The *FokI* rs2228570 TC/CC genotypes (OR = 1.77, 95%CI 1.2-2.7, p=0.007) and age ≥55 years (OR=2.28; 95%CI: 1.55-3.37, p<0.001) were independently associated with advanced liver fibrosis. *VDR* polymorphisms were not associated with pre-treatment serum HCV RNA. In Thai patients with chronic HCV infection, the bAt haplotype is associated with poor response to PEG-IFN-

based therapy, and the *FokI* rs2228570 TC/CC genotypes are risk factors for advanced liver fibrosis.

Genetic Associations of Vitamin D Receptor Polymorphisms with Advanced Liver Fibrosis and Response to Pegylated Interferon-based Therapy in Chronic Hepatitis C

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27 **Abstract**

28 Vitamin D receptor (VDR) modulates host immune responses, including interferon signaling. This
29 study aimed to investigate the associations of *VDR* polymorphisms with advanced liver fibrosis
30 and response to pegylated interferon (PEG-IFN)-based therapy in patients with chronic hepatitis
31 C virus (HCV) infection. In total, 554 Thai patients with chronic HCV infection treated with a
32 PEG-IFN-based regimen were enrolled. Six single-nucleotide polymorphisms (SNPs) were
33 genotyped: the *IL28B* C>T (rs12979860) SNP and five *VDR* SNPs, comprising *FokI* T>C
34 (rs2228570), *BsmI* C>T (rs1544410), *Tru9I* G>A (rs757343), *ApaI* C>A (rs7975232), and *TaqI*
35 A>G (rs731236). In total, 334 patients (60.3%) achieved sustained virological response (SVR),
36 and 255 patients (46%) were infected with HCV genotype 1. The bAt (CCA) haplotype, consisting
37 of the *BsmI* rs1544410 C, *ApaI* rs7975232 C, and *TaqI* rs731236 A alleles, was associated with
38 poor response to PEG-IFN-based therapy. The *IL28B* rs12979860 CT/TT genotypes (OR=3.66,
39 95%CI: 2.26-5.91, p<0.001), bAt haplotype (OR=2.12, 95%CI: 1.11-4.05, p=0.02), pre-treatment
40 serum HCV RNA (logIU/ml; OR=1.75, 95%CI: 1.33-2.31, p<0.001), and advanced liver fibrosis
41 (OR=1.60, 95%CI: 1.05-2.44, p=0.03) independently predicted poor response. Patients with the
42 bAt haplotype were more likely to have poor response compared to patients with other haplotypes
43 (41.4% vs 21.9%, p=0.03). The *FokI* rs2228570 TC/CC genotypes (OR = 1.77, 95%CI 1.2-2.7,
44 p=0.007) and age ≥55 years (OR=2.28; 95%CI: 1.55-3.37, p<0.001) were independently
45 associated with advanced liver fibrosis. *VDR* polymorphisms were not associated with pre-
46 treatment serum HCV RNA. In Thai patients with chronic HCV infection, the bAt haplotype is
47 associated with poor response to PEG-IFN-based therapy, and the *FokI* rs2228570 TC/CC
48 genotypes are risk factors for advanced liver fibrosis.

49 **Subjects** Genetics, Gastroenterology and Hepatology, Translational Medicine

50 **Key words** Vitamin D receptor polymorphisms, hepatitis C virus, pegylated interferon, advanced
51 liver fibrosis

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major health problem affecting >71.1 million people worldwide, leading to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (Organization 2017). The advancement of HCV treatment in terms of the development of direct-acting antiviral agents (DAAs) has evoked international interest in the global elimination of HCV. In 2017, the World Health Organization set targets to eliminate globally viral hepatitis as a public threat by 2030 by achieving a 90% diagnosis rate, an 80% treatment rate, and a 65% reduction in the mortality rate. However, in low- and middle-income countries (including those in Southeast Asia, the Middle East, and North Africa), which have 80% of the global HCV burden, access to DAAs is constrained by financial factors (Jayasekera et al. 2014; Mohd Hanafiah et al. 2013; Zoulim et al. 2015). Pegylated interferon (PEG-IFN)-based therapy is still being used in these countries. The current Asian Pacific Association for the Study of the Liver (APASL) guidelines on the treatment of HCV infection continue to recommend PEG-IFN and ribavirin as first-line therapy in resource-limited countries where DAAs are unavailable (Omata et al. 2016).

Current evidence shows that in addition those in supporting calcium and bone metabolism, vitamin D (VD) plays several important roles in immunomodulation, regulation of cellular proliferation, differentiation, and apoptosis (Holick 2007; Penna et al. 2005; von Essen et al. 2010). Several studies have reported association between VD deficiency and risk of cancer, congestive heart failure, insulin resistance, and autoimmune diseases (Feskanich et al. 2004; Giovannucci et al. 2006; Munger et al. 2006). The liver is a crucial organ in VD synthesis as it is the site of the enzymatic conversion of the inactive form of VD to 25-dihydroxyVD. VD deficiency was found in 70% of patients with chronic liver disease regardless of the etiology, and 22% had severe VD deficiency (Arteh et al. 2010). Patients with chronic HCV infection had lower serum VD levels than sex- and age-matched healthy controls (Petta et al. 2010). In terms of clinical outcomes, low VD level is probably independently related to advanced liver fibrosis and high necroinflammatory activity in chronic HCV patients (Dadabhai et al. 2017; Petta et al. 2010). Two large meta-analyses reported a negative association between sustained virological response (SVR) and VD level in chronic HCV patients treated with PEG-IFN therapy (Garcia-Alvarez et al. 2014; Villar et al. 2013).

Vitamin D receptor (VDR) is a nuclear hormone receptor that can act as a ligand-inducible transcription factor. VDR binds to the active form of VD and thereby mediates its effect (Keane et al. 2018). VDR is expressed in many cells of the human body, including monocytes and activated T lymphocytes in the peripheral blood, non-parenchymal cells (hepatic stellate cells, Kupffer cells, and endothelial cells), and hepatocytes, especially during inflammatory liver injury (Gascon-Barre et al. 2003; Keane et al. 2018). The receptor is encoded by the *VDR* gene, which is located on chromosome 12q. The gene has a promoter, regulatory regions, and exons 2–9, which span over 100 kb (Deeb et al. 2007; Uitterlinden et al. 2004). Using different restriction endonucleases for *BsmI*, *Tru9I*, *ApaI*, and *TaqI* (to cleave the DNA at the 3′ end) and *FokI* (to cleave the DNA in exon 2), multiple *VDR* polymorphisms have been explored (Uitterlinden et al. 2004). The bAt (CCA) haplotype is a common genetic variant of the *VDR* gene, comprising the following three polymorphisms at the 3′ end of the gene: *BsmI* rs1544410 C, *ApaI* rs7975232 C,

103 and *TaqI* rs731236 A, which are in strong linkage disequilibrium. Recent research shows that *VDR*
104 genetic variations lead to susceptibility and chronicity regarding HCV infection (Wu et al. 2016).
105 In addition, *VDR* polymorphisms may be related to the response to PEG-IFN and ribavirin therapy
106 in chronic HCV patients. However there have been conflicting results regarding these relationships
107 among previous studies (Baur et al. 2012b; Garcia-Martin et al. 2013; Hung et al. 2016; Shaker et
108 al. 2016). This study aims to investigate whether the common *VDR* polymorphisms are associated
109 with the response to PEG-IFN-based therapy and advanced liver fibrosis in patients with chronic
110 HCV infection.

MATERIALS & METHODS

Patients

113   This study included Thai patients with chronic HCV infection at Chulalongkorn University
114 hospital (Bangkok, Thailand) and Srinagarind hospital (Khon Kaen, Thailand) from June 2012 to
115 December 2013. All patients were seropositive for anti-HCV antibody and HCV RNA and they
116 were treated with PEG-IFN and ribavirin in accordance with standard guideline recommendations
117 (European Association for the Study of the 2011; Ghany et al. 2009). Patients who had co-infection
118 with hepatitis B virus or human immunodeficiency virus, decompensated cirrhosis, or liver
119 transplants were excluded. Baseline characteristics were recorded, and biochemical and virological
120 tests were conducted at baseline during treatment and at 24 weeks after treatment. Liver biopsy or
121 the Fibrosis-4 (Fib-4) index were used for assessing liver fibrosis. Advanced liver fibrosis was
122 defined as fibrosis stage 3–4 by histology or Fib-4 index > 3.25 (Vallet-Pichard et al. 2007).

123   The study followed the principles of the Declaration of Helsinki and was approved by the
124 local Institutional Review Board (IRB) committee on medical ethics. Written informed consent
125 was obtained from each participant.

Virological testing

127   The quantitative serum HCV RNA level was evaluated using the real-time polymerase
128 chain reaction (RT-PCR) COBAS® Taqman® HCV test (Roche Diagnostics, Basel, Switzerland).
129 HCV genotyping was performed using the INNO-LiPA HCV II assay (Innogenetics, Ghent,
130 Belgium).

Genotyping

132   Genotyping of the following six single-nucleotide polymorphisms (SNPs) was performed:
133 the interleukin 28B (*IL28B*; also known as interferon lambda 3 [IFNl3]) C>T (rs12979860) SNP
134 and five *VDR* SNPs, comprising *FokI* T>C (rs2228570), *BsmI* C>T (rs1544410), *Tru9I* G>A
135 (rs757343), *ApaI* C>A (rs7975232), and *TaqI* A>G (rs731236). DNA was extracted from 100 μL
136 of peripheral blood leukocytes using a standard phenol-chloroform protocol and then kept at −
137 80°C. Next, 2 μL DNA was subjected to PCR (total volume, 25 μL) using Perfect *Taq* Plus
138 MasterMix (5 PRIME GmbH, Hamburg, Germany). The PCR-specific probes and conditions are
139 summarized in Supplementary Table 1. This was followed by restriction fragment length
140 polymorphism (RFLP) assays except genotyping *IL28B* was done by sequencing method (First

141 BASE Laboratories, Selangor, Malaysia). Subsequently, 2% agarose gel electrophoresis was used
142 to assess the resulting DNA fragments. The separated DNA was viewed under ultraviolet light
143 after staining with ethidium bromide.

144 Three of the SNPs located at the 3′ end of the *VDR* gene (*BsmI*, *ApaI*, and *TaqI*) are in
145 strong linkage disequilibrium, and the bAt (CCA) haplotype involves *BsmI* rs1544410 C, *ApaI*
146 rs7975232 C, and *TaqI* rs731236 A.

Statistical analysis

148 Statistical analysis was performed using SPSS version 22.0 (IBM Corp., Armonk, NY,
149 USA). Categorical data are expressed as number (percentage), and the differences between groups
150 were compared using the chi-square test. Continuous data are expressed as mean ± standard
151 deviation, and the differences between groups were compared using Student's t-test and the Mann–
152 Whitney *U* test. The effects of pre-treatment factors and the SNPs on the response to PEG-IFN-
153 based therapy (in term of SVR) and the presence of advanced liver fibrosis were investigated using
154 univariate and stepwise multivariate logistic regression analyses. A *p*-value <0.05 was considered
155 statistically significant. The chi-square test was used to verify whether the genotype frequencies
156 related to the SNPs in patients with and without SVR were in accordance with the Hardy–
157 Weinberg assumption.

RESULTS

Patient characteristics

160 A total of 554 Thai patients with chronic HCV infection were enrolled. There were 365
161 men (65.9%) and the mean age was 50.9 ± 9.2 years. Three hundred and thirty-four patients
162 (60.3%) achieved SVR, 255 patients (46%) were infected with HCV genotype 1, and 179 patients
163 (32.3%) had advanced liver fibrosis. Table 1 shows the participants' baseline demographic and
164 laboratory data according to treatment response at 24 weeks after PEG-IFN discontinuation.
165 Compared to patients with poor response, patients who achieved SVR were older, had lower pre-
166 treatment serum HCV RNA levels, and were less likely to have HCV genotype 1, advanced liver
167 fibrosis, and the unfavorable *IL28B* rs12979860 CT/TT genotypes.

Prevalence of VDR *polymorphisms and bAt (CCA) haplotype and their associations with response to PEG-IFN-based therapy*

170 The frequencies of the *VDR* genotypes and the bAt (CCA) haplotype and their associations
171 with response to PEG-IFN-based therapy are shown in Table 2. The genotypic frequencies of the
172 SNPs were in Hardy–Weinberg equilibrium (p>0.05) except for *Tru9I* (rs757343). The genotypic
173 frequencies of the SNPs were not different between patients with and without SVR.

174 The *FokI*, *BsmI*, *Tru9I*, *ApaI*, and *TaqI* polymorphisms were not associated with response
175 to PEG-IFN-based therapy. However, the bAt (CCA) haplotype was significantly associated with
176 poor response to PEG-IFN-based therapy. Overall, 41.4% of patients with the bAt (CCA)
177 haplotype were poor responders, resulting in an OR of 1.82 (95% CI: 1.04-3.18, p=0.03) when
178 compared to patients who had other haplotypes (27.9%).

179 ***Factors associated with response to PEG-IFN-based therapy***

180   Based on univariate analysis, advanced age, HCV genotype 1, high pre-treatment HCV
181 RNA level, advanced liver fibrosis, *IL28B* rs12979860 CT/TT, and the bAt (CCA) haplotype were
182 significantly associated with poor response to PEG-IFN-based therapy. Stepwise multivariate
183 regression analysis showed that the *IL28B* rs12979860 CT/TT genotypes (OR=3.66, 95%CI: 2.26-
184 5.91, p<0.001), the bAt (CCA) haplotype (OR=2.12, 95%CI: 1.11-4.05, p=0.02), pre-treatment
185 HCV RNA level (logIU/ml; OR=1.75, 95%CI: 1.33-2.31, p<0.001), and advanced liver fibrosis
186 (OR=1.60, 95%CI: 1.05-2.44, p=0.03) were independent baseline predictors of poor response to
187 PEG-IFN-based therapy.

188 ***Comparison between bAt (CCA) and other haplotypes***

189   Among the participants, 486 (87.7%) had the bAt (CCA) haplotype. To explore the
190 mechanisms underlying the association between this haplotype and poor response to PEG-IFN-
191 based therapy, the baseline patient characteristics, virological factors, and liver fibrosis stage were
192 compared between the chronic HCV patients with the bAt (CCA) haplotype and any other
193 haplotypes (Table 3). There were no differences in sex, age, body mass index, diabetes mellitus,
194 HCV genotype, pre-treatment HCV RNA level, advanced liver fibrosis, or rapid or early
195 virological response. However, patients with the bAt (CCA) haplotype were more likely to drink
196 alcohol and have unfavorable *IL28* rs12979860 CT/TT genotypes, and had lower pre-treatment
197 alanine Aminotransferase (ALT) levels than patients with other haplotypes.

198 ***Advanced liver fibrosis and HCV RNA level according to* VDR *polymorphisms***

199   Five hundred and ten patients (92.1%) had data on liver fibrosis stage. Of these, 269
200 (52.7%) underwent liver biopsy before treatment with PEG-IFN and ribavirin, and 179 patients
201 (52.3%) were diagnosed with advanced liver fibrosis. Figure 1 shows the percentage of advanced
202 liver fibrosis according to each *VDR* genotype. Chronic HCV patients with the *FokI* rs2228570
203 TC/CC genotypes (38.8%) were more likely to have advanced liver fibrosis compared to patients
204 with the CC genotype (26.3%). The associations between the *VDR* genotypes and pre-treatment
205 HCV RNA level are shown in Figure 2. Pre-treatment HCV RNA level was not significantly
206 different among patients who had different *VDR* genotypes.

207 ***Factors associated with advanced liver fibrosis***

208   Univariate and multivariate analysis results for advanced liver fibrosis are shown in Table
209 4. Based on the univariate analysis, advanced liver fibrosis (assessed by liver histology or FIB-4
210 index) was associated with age ≥55 years (p<0.001), being male (p=0.08) and *FokI* TC/CC
211 genotypes (p=0.007). Factors with p<0.1 in the univariate analysis were included in the
212 multivariate model. Based on the multivariate analysis, age ≥55 years (OR=2.28; 95%CI: 1.55-
213 3.37, p<0.001) and *FokI* TC/CC genotypes (OR=1.58; 95%CI: 1.03-2.43, p=0.03) were
214 independent predictors of advanced liver fibrosis. The *BsmI*, *Tru9I*, *ApaI*, and *TaqI* genotypes, and
215 the bAt (CCA) haplotype were not associated with advanced liver fibrosis.

216 **DISCUSSION**

217   The main findings are that the *FokI* rs2228570 TC/CC genotypes are independently
218   associated with an increased risk of advanced liver fibrosis in Thai chronic HCV patients.
219   Additionally, the *VDR* bAt (CCA) haplotype was independently associated with poor response to
220   PEG-IFN and ribavirin in patients with chronic HCV infection. Interestingly, these associations
221   did not depend on the unfavorable *IL28B* rs12979860 CT/TT genotypes, HCV genotype, or pre-
222   treatment HCV viral load. This study provides evidence indicating the important effects of *VDR*
223   polymorphisms on clinical outcomes in patients with chronic HCV infection.

224   VDR acts as a ligand-induced transcription factor that binds to 1, 25-dihydroxyVD and
225   exerts its effects by regulating the expression of >900 genes in target tissues (Kato 2000). Recent
226   studies have indicated that 1, 25-dihydroxyVD and VDR are important regulators of both the
227   innate and adaptive immune response (Khammissa et al. 2018; Rosen et al. 2012). VDR expression
228   is exhibited in almost all immune cells including B cells, activated T lymphocytes, neutrophils,
229   natural killer cells, and antigen-presenting cells (Bhalla et al. 1983; Provvedini et al. 1983). The 1,
230   25-dihydroxyVD/VDR signaling pathway can activate monocytes, inhibit lymphocyte
231   proliferation, and prevent the differentiation of dendritic cell precursors into antigen-presenting
232   cells (Berer et al. 2000). In addition, 1, 25-dihydroxyVD is able to suppress *IFN-γ* transcription
233   via the binding of VDR to a silencer region in the *IFN-γ* gene promoter (Saggese et al. 1989).
234   Genetic variations of the *VDR* gene can result in a dysfunctional receptor that subsequently affects
235   the function of VD. *VDR* polymorphisms have been implicated in susceptibility to a variety of
236   autoimmune diseases and cancers in a genome-wide association study and meta-analysis
237   (Raimondi et al. 2009; Ramagopalan et al. 2010). Interestingly, *VDR* gene variants regulate the
238   biological effects of VD independently of the serum 1, 25-dihydroxyVD level (Khammissa et al.
239   2018).

240   Regarding the association between VDR and response to PEG-IFN-based therapy in
241   chronic HCV infection, a recent *in vitro* study reported that 1, 25-dihydroxyVD promotes the
242   inhibitory effect of IFN-α on HCV replication by enhancing the expression of IFN-stimulated
243   genes (ISGs) [39]. The crosstalk between VDR and IFN-α signaling may help to better understand
244   the underlying mechanisms in clinical studies of HCV infection. The results from the current study
245   showed that the *VDR* bAt (CCA) haplotype was associated with poor response to PEG-IFN-based
246   therapy in Thai patients with chronic HCV infection. Although this association has been reported
247   in several previous studies, the findings have been conflicting. Baur *et al*. and García-Martín *et al*.
248   reported that Caucasian patients with chronic HCV infection with the bAt (CCA) haplotype had
249   an impaired response to PEG-IFN and ribavirin [23, 25]. In contrast, Hung *et al*. did not find an
250   association between the bAt (CCA) haplotype and antiviral response to PEG-IFN therapy in 139
251   Taiwanese patients with chronic HCV genotype-1 infection [22]. The possible reason for the
252   discordant results between the two studies in Asian chronic HCV patients (i.e., our study and the
253   Hung *et al*. study) may be the lower prevalence of the bAt haplotype in the previous study (54.7%)
254   compared to our study (87.7%). The mechanism underlying the association between the bAt
255   (CCA) haplotype and poor response to PEG-IFN is still unclear. It may be due to the effect of this
256   haplotype on the immune response-related IFN signaling cascade [36], as we found no relationship
257   between the bAt (CCA) haplotype and pre-treatment HCV RNA level or liver fibrosis stage.
258   Additionally, our study did not find any relationships between the *VDR* SNPs and the response to

259 PEG-IFN-based therapy. In contrast, previous studies reported negative associations between the
260 response to PEG-IFN-based therapy and both the *FokI* T allele [23, 40, 41] and the *TaqI* G allele
261 [41] in patients with chronic HCV infection.

262   With regard to the relationship between *VDR* polymorphisms and clinical outcomes, an *in*
263 *vitro* analysis showed that VDR ligands inhibited transforming growth factor (TGF)-β1-induced
264 hepatic stellate cell activation and reduced liver fibrosis, while, in a mouse model, genetic
265 knockout of VDR expression led to spontaneous liver fibrosis (Ding et al. 2013). The response of
266 human hepatic stellate cells to TGF-β1 and VD partially depends on the *VDR* polymorphisms
267 (Beilfuss et al. 2015). In patients with chronic HCV genotype 1 infection, low serum VD level is
268 related to severe liver fibrosis (Petta et al. 2010). VDR expression is exhibited in hepatic
269 parenchymal and inflammatory cells of patients with chronic HCV infection, and low VDR
270 expression is associated with high portal inflammation (Barchetta et al. 2012). The current study
271 showed the *VDR FokI* rs2228570 TC/CC genotypes and age ≥55 years were independent risk
272 factors for advanced liver fibrosis in Thai patients with chronic HCV infection. The *BsmI*, *Tru9I*,
273 *ApaI*, and *TaqI* polymorphisms, and the bAt (CCA) haplotype were not associated with advanced
274 liver fibrosis or pre-treatment HCV RNA level in our cohort. Previous research reported that *VDR*
275 variants might be related to decrease HCV infection susceptibility in a Chinese population (Wu et
276 al. 2016). A cohort study of Swiss chronic HCV patients by Baur *et al.* showed the bAt (CCA)
277 haplotype was associated with rapid fibrosis progression and cirrhosis (Baur et al. 2012a). *BsmI*
278 and *TaqI* polymorphisms were associated with liver fibrosis in a Brazilian cohort in a study by
279 Scalioni *et al.* (Scalioni et al. 2018). In Taiwanese patients with chronic HCV infection, the bAt
280 (CCA) haplotype, *ApaI* CC genotype, and *TaqI* AA genotype were associated with increased HCV
281 RNA levels compared to other genotypes/haplotypes (Hung et al. 2016). Furthermore, the *ApaI*
282 CC genotype was an independent factor for the development of HCC in a Taiwanese HCV cohort
283 (Hung et al. 2014).

284   The *FokI* polymorphism restriction site is located in exon 2 in the 5′ coding region of *VDR*.
285 This polymorphism leads to a T>C (threonine to cysteine) substitution and the generation of a
286 protein shortened by three amino acids, which makes the protein less functionally active than the
287 wild type (van Etten et al. 2007). This polymorphism has been implicated in the response to PEG-
288 IFN therapy and several chronic liver diseases including autoimmune hepatitis and HCC in patients
289 with chronic HBV infection (Mostafa-Hedeab et al. 2018; Vogel et al. 2002; Yao et al. 2013). The
290 present study found an association between the *FokI* polymorphism and advanced liver fibrosis in
291 patients with chronic HCV infection. The *FokI* polymorphism genotypic frequencies in a healthy
292 Thai population have been reported to be 15.7% for TT, 43.6% for TC, and 40.7% for CC
293 (Sangkaew et al. 2018). These frequencies are consistent with our results showing frequencies of
294 20.9% for TT, 48.9% for TC, and 30.2% for CC in Thai patients with chronic HCV infection.

295   Our study had several limitations. We did not investigate the relationship between the
296 baseline serum VD level and response to PEG-IFN-based therapy because it is influenced by many
297 confounding factors such as sunlight exposure, nutritional status, and liver function. In addition,
298 *VDR* variants can modulate their effects independently of serum VD status (Uitterlinden et al.
299 2004). Additionally, our study was a retrospective study, and pre-treatment serum samples were

300 not collected for most of the participants. However, we still attempted to assess associations
301 between the *VDR* variants and clinical outcomes in patients with chronic HCV infection.

CONCLUSIONS

303 The present study demonstrates an association between the *VDR* bAt (CCA) haplotype and
304 poor response to PEG-IFN plus ribavirin therapy and associations between the *VDR FokI*
305 rs2228570 TC/CC genotypes and advanced liver fibrosis in Thai patients with chronic HCV
306 infection. These results provide helpful clinical information for understanding the causation
307 between *VDR* polymorphisms and clinical outcomes. Further studies are required to elucidate the
308 detailed molecular mechanisms.

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319 **Conflict of interest**

320 The authors declare there are no competing interests.

321 **Author Contributions**

322 • Kessarin Thanapirom conceived and designed the experiments, analyzed the data, prepared
323   figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
324 • Sirinporn Suksawatamnuay performed the experiments, analyzed the data, approved the
325   final draft.
326 • Wattana Sukeepaisarnjaroen contributed sample of patients/reagents/materials/analysis,
327   approved the final draft.

328 • Pisit Tangkijvanich contributed sample of patients/reagents/materials/analysis, approved
329   the final draft.

330 • Panarat Thaimai performed the experiments, approved the final draft.

331 • Rujipat Wasitthankasem performed the experiments, approved the final draft.

332 • Yong Poovorawan contributed reagents/materials/analysis tools, authored or reviewed
333   drafts of the paper, approved the final draft.

334 • Piyawat Komolmit conceived and designed the experiments, contributed
335   reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed
336   drafts of the paper, approved the final draft.

337 **Ethics**

338 The study protocol was approved by the Institutional Review Board of the Faculty of Medicine,
339 Chulalongkorn University (IRB number 562/54) and Khon Kaen University (HE561177).

340 **Data Availability**

341 The following information was supplied regarding data availability:

342 The raw data files are available in Supplemental Files: VDR1

343 **Supplemental Information**

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Baseline patient characteristics according to response to PEG-IFN-based therapy

1 **Table 1: Baseline patient characteristics according to response to PEG-IFN-**
2 **based therapy**

	Non-SVR (n=220)	SVR (n=334)	p-value
Female, n (%)	71 (32.3%)	118 (35.3%)	0.46
Age (years), mean ± SD	52.1 ± 8.0	50.1 ± 9.8	0.01
Body mass index (kg/m^2), mean ± SD	24.6 ± 3.4	24.6 ± 3.6	0.96
Alcohol drinking, n (%)	134 (69.8%)	142 (61.7%)	0.1
Diabetes Mellitus, n (%)	51 (26.2%)	53 (22.7%)	0.41
Genotype, n (%)			
1	122 (55.5%)	133 (39.8%)	<0.001
2	0	1 (0.3%)	
3	85 (38.6%)	160 (47.9%)	
6	13 (5.9%)	40 (12%)	
HCV RNA (log IU/ml), mean ± SD	6.05 ± 0.61	5.8 ± 0.8	0.002
ALT (U/L), mean ± SD	107.5 ± 166.5	100.1 ± 74.0	0.49
Advanced liver fibrosis, n (%)	84 (41.8%)	95 (30.7%)	0.01
IL28B rs12979860, n (%)			
CC	148 (67.3%)	290 (86.8%)	<0.001
CT	69 (31.4%)	38 (11.4%)	
TT	3 (1.4%)	6 (1.8%)	

Frequencies of the *VDR* genotypes and the bAt haplotype in Thai patients with chronic hepatitis C infection treated with PEG-IFN.

1 **Table 2: Frequencies of the *VDR* genotypes and the bAt haplotype in Thai**
2 **patients with chronic hepatitis C infection treated with PEG-IFN.**

	All patients (n=554)	Non-SVR (n=220)	SVR (n=334)	Odds ratio (95% CI)	p-value
FokI rs2228570					
TT	116 (20.9%)	51 (23.2%)	65 (19.5%)	0.80 (0.53-1.21)	0.29
TC	271 (48.9%)	105 (47.7%)	166 (49.7%)		
CC	167 (30.2%)	64 (29.1%)	103 (30.8%)		
BsmI rs1544410					
CC	453 (81.8%)	181 (82.3%)	272 (81.4%)	0.95 (0.61-1.47)	0.80
CT	94 (17.0%)	36 (16.4%)	58 (17.4%)		
TT	7 (1.3%)	3 (1.4%)	4 (1.2%)		
Tru9I rs757343					
GG	326 (58.8%)	136 (61.8%)	190 (56.9%)	0.82 (0.58-1.15)	0.25
GA	197 (35.6%)	74 (33.6%)	123 (36.8%)		
AA	31 (5.6%)	10 (4.5%)	21 (6.3%)		
ApaI rs7975232					
CC	252 (45.5%)	106 (48.2%)	146 (43.7%)	0.84 (0.59-1.18)	0.30
CA	240 (43.3%)	95 (43.2%)	145 (43.4%)		
AA	62 (11.2%)	19 (8.6%)	43 (12.9%)		
TaqI rs731236					
AA	477 (86.1%)	197 (89.5%)	280 (83.8%)	0.61 (0.36-1.02)	0.06
AG	68 (12.3%)	23 (10.5%)	45 (13.5%)		

GG	9 (1.6%)	0	9 (2.7%)		
bAt (CCA) haplotype	486 (87.7%)	201 (91.4%)	285 (85.3%)	1.82 (1.04-3.18)	0.03

Baseline characteristics, virological factors and liver fibrosis stage in accordance to the bAt (CCA) haplotype.

1 **Table 3: Baseline characteristics, virological factors and liver fibrosis stage in**
2 **accordance to the bAt (CCA) haplotype.**

	CCA haplotype (n=486)	Other haplotypes (n=68)	p-value
Female, n (%)	160 (32.9%)	29 (42.6%)	0.11
Age (years), mean ± SD	50.8 ± 9.05	51.5 ± 10.3	0.57
Body mass index (kg/m²), mean ± SD	24.5 ± 3.5	25.3 ± 3.3	0.88
Alcohol drinking, n (%)	255 (67.5%)	21 (47.7%)	0.01
Diabetes mellitus, n (%)	9 (23.2%)	15 (34.1%)	0.11
HCV genotype 1, n (%)	229 (47.1%)	26 (38.2%)	0.17
Pre-treatment HCV RNA level (log IU/ml), mean ± SD	5.9313 ± 0.77	5.8724 ± 0.69	0.57
Pre-treatment ALT, mean ± SD	97.3 ± 68.0	143.5 ± 285.9	0.004
Advanced liver fibrosis, n (%)	160 (35.7%)	19 (30.6%)	0.43
Rapid virological response, n (%)	257 (65.6%)	37 (71.2%)	0.42
Early virological response, n (%)	371 (88.8%)	48 (87.3%)	0.75
IL28B rs12979860 CT/TT genotypes, n (%)	93 (19.1%)	23 (33.8%)	0.005

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Univariate and multivariate regression analyses of factors associated with advanced liver fibrosis in patients with chronic HCV infection.

1   **Table 4: Univariate and multivariate regression analyses of factors associated**
2   **with advanced liver fibrosis in patients with chronic HCV infection.**

	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age ≥ 55 years	2.48 (1.69-3.62)	<0.001	2.28 (1.55-3.37)	<0.001
Male	1.40 (0.96-2.05)	0.08	1.19 (0.80-1.77)	0.39
Body mass index	1.01 (0.96-1.07)	0.62		
Alcohol consumption	1.00 (0.65-1.55)	0.99		
HCV genotype 1	0.77 (0.53-1.11)	0.17		
IL28B rs12979860 CT/TT genotypes	0.92 (0.59-1.45)	0.72		
FokI rs2228570 TC/CC genotypes	1.78 (1.17-2.70)	0.007	1.58 (1.03-2.43)	0.03
BsmI rs1544410 GG genotype	0.98 (0.61-1.57)	0.92		
Tru9l rs757343 GG genotype	1.04 (0.72-1.50)	0.85		
Apal rs7975232 GG genotype	0.90 (0.62-1.30)	0.57		
TaqI rs731236 TT genotype	0.83 (0.49-1.41)	0.50		
bAt (CCA) haplotype	1.26 (0.71-2.23)	0.43		

Figure 1

Association between advanced liver fibrosis and *VDR* polymorphisms in patients with chronic HCV infection.
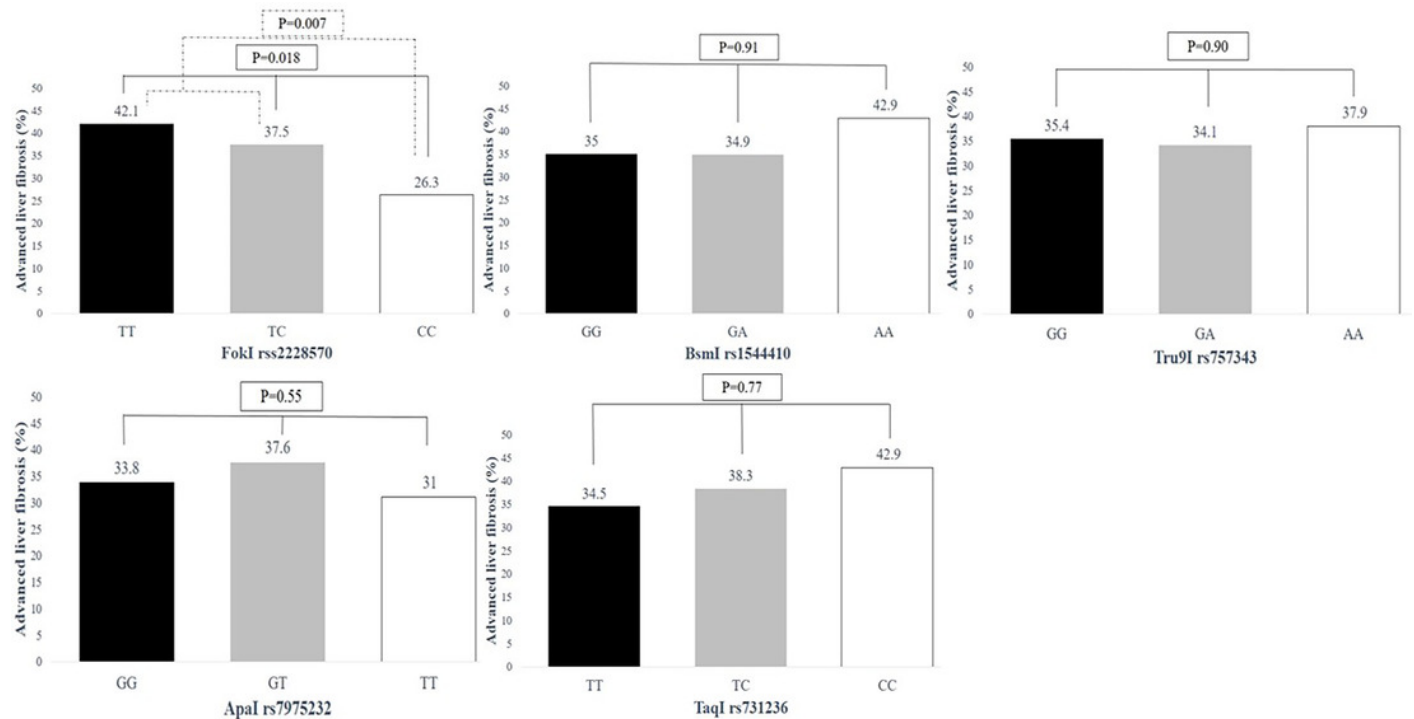
Figure 2

Baseline serum HCV RNA according to *VDR* polymorphisms in patients with chronic HCV infection