Pakistan J. Zool., pp 1-6, 2021. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20210909030800

ApaI VDR Polymorphism as a Risk Factor of Treatment Failure in Chronic Hepatitis C Patients

Noora Hassan Hezam Al-Aqmer[1]*, Zain Aamir[2], Muhammad Farooq Hanif[3], Soumble Zulfiqar[4], Sibgha Zulfiqar[1], Mateen Izhar[5] and Abdul Rauf Shakoori[4]*

[1]Department of Physiology, Shaikh Zayed Postgraduate Medical Institute, Shaikh Zayed Medical Complex, Lahore, Pakistan
[2]Primary and Secondary Healthcare Department, Lahore, Pakistan
[3]Gastroenterology and Hepatology, Pakistan Kidney and Liver Institute and Research Center, Lahore, Pakistan
[4]School of Biological Sciences, University of the Punjab, Lahore, Pakistan
[5]Department of Microbiology, Shaikh Zayed Postgraduate Medical Institute, Shaikh Zayed Medical Complex, Lahore, Pakistan

Article Information
Received 12 June 2021
Revised 19 October 2021
Accepted 27 October 2021
Available online 16 November 2021

Authors' Contribution
NHHA designed the study, collected the data, did genetic analysis, statistical analysis, and wrote the manuscript. ZA participated in analysis. MFH participated in data collection. SZ supervised the genetic analysis work and reviewed the manuscript. MI reviewed the manuscript. ARS reviewed the study and the manuscript critically.

Key words
Vitamin D receptor polymorphism, Chronic hepatitis C, *ApaI* VDR polymorphism

ABSTRACT

The objective of this study was to find out the association between *ApaI* vitamin D receptor (VDR) polymorphism and the response to hepatitis C directly acting antiviral treatment. This study which is a case control study included 66 hepatitis C patients (genotype 3) who responded to the directly acting antiviral treatment and achieved negative HCV-RNA three months after completing the treatment (sustained virologic response (SVR)) and 66 hepatitis C patients (genotype 3) who did not achieve SVR three months after completing the same treatment. Informed consent was taken from participants, demographic data was collected, and 5 mL of blood was drawn from each participant and used for DNA extraction, polymerase chain reaction and restriction fragment length polymorphism analysis. After restriction, samples were run on 2% agarose gel followed by visualization under UV light. Data analysis was done using IBM SPSS 24. We found that the distribution of *ApaI* genotypes was 28 (42.4%), 27 (40.9%), and 11 (16.7%) for the genotypes AA, Aa, and aa in responders and 22 (33.3%), 26 (39.4%), and 18 (27.3%) in non-responders. The allelic distribution was 83 (62.9%) and 49 (37.1%) for the "A" and "a" alleles in responders and 70 (53%) and 62 (47%) in non-responders. *ApaI* genotype ''aa" was found to be a significant predictor of treatment failure (p-value= .024, OR= 3.589, 95% CI= 1.181-10.911). There was no significant association between *ApaI* VDR genotypes and cirrhosis and *ApaI* VDR genotypes and gender (p-values < .05). To conclude *ApaI* genotype aa could be used as a marker to predict treatment failure in hepatitis C patients receiving directly acting antiviral treatment.

INTRODUCTION

Hepatitis C virus is an important world-wide health problem (Stanaway *et al*., 2016). It is a main cause of cirrhosis, hepatocellular carcinoma, and mortality (Perz *et al*., 2006). Hepatitis C virus is also shown to cause complications beyond the liver such as lymphoma, diabetes, and chronic renal disease (Younossi *et al*., 2016). It is estimated that approximately 71 million individuals are infected with hepatitis C virus worldwide (Polaris Observatory HCV Collaborators, 2017). More than 50% of HCV infections are in China, Pakistan, Egypt, Nigeria, Russia, and India (Gower *et al*., 2014).

In Pakistan, the prevalence of hepatitis C virus is the 2nd highest in the world (Hill *et al*., 2017; Abbas and Abbas, 2020) and is about 5% nationwide (Al Kanaani *et al*., 2018) which is persistently high without evidence of a decline since three decades (Mahmud *et al*., 2019) and the prevalence in rural areas and peri-urban areas is up to 25% (Umer and Iqbal, 2016). In Pakistan, genotype 3a is common (Haqqi *et al*., 2019) and to achieve WHO target of elimination of hepatitis C by 2030, treatment has to be provided to a million hepatitis C infected patients yearly (Altaf and Pasha, 2020).

Vitamin D receptors are hormonal receptors in the nucleus of the cell. They are ligand-activated regulatory proteins that direct the transcription machine to specific genomic sites to influence RNA production and therefore encoding proteins which are important for specific biological functions (Pike and Meyer, 2012). Vitamin D receptor is involved in different physiological processes

* Corresponding author: arshakoori.sbs@pu.edu.pk, drnooraalaqmer@gmail.com
0030-9923/2021/0001-0001 $ 9.00/0

and that include cell differentiation and proliferation and immune modulation (Adams and Hewison, 2008).

Several vitamin D receptor single nucleotide polymorphisms were found to be associated with the risk of hepatitis C infection (Wu *et al*., 2016), progression of the disease (Baur *et al*., 2012) as well as with the response to treatment (Garcia-Martin *et al*., 2013; Al-Aqmer *et al*., 2021). Baur *et al*. (2012) found *ApaI* vitamin D receptor polymorphism to be inversely associated with the response to pegylated interferon with ribavirin and considered it as risk factor for failure of treatment. However, there were studies which reported no association between *ApaI* vitamin D receptor polymorphism and the response to treatment (Arai *et al*., 2015; Abdelsalam *et al*., 2016; Wang *et al*., 2016; Thanapirom *et al*., 2019).

As no study was conducted specifically on hepatitis C genotype 3 patients receiving directly acting antiviral treatment, this study aimed to find out the association of *ApaI* vitamin D receptor polymorphism with the response to directly acting antiviral treatment in hepatitis C genotype 3 Pakistani patients.

MATERIALS AND METHODS

This case control study was conducted after approval from the Ethical Review Board of Pakistan Kidney and Liver Institute, Lahore and included 66 responders who received hepatitis C antiviral treatment, daclatasvir and sofosbuvir (with ribavirin in case of cirrhotic patients), and achieved a sustained virologic response (HCV-RNA negative) three months after completing the treatment, males and females ≥ 18 years in age, and were matched in age and gender with 66 non-responders who received the same treatment and did not achieve a sustained virologic response (HCV-RNA positive) three months after completing the treatment. Patients with advanced liver disease, renal disease, hepatitis B, and HIV were excluded. After written consent was taken from the participants, demographic data and reports of hemoglobin, liver function tests, platelet count, prothrombin time, and INR were recorded. About 5 mL of blood was drawn for DNA extraction followed by polymerase chain reaction (PCR) of the DNA sequence containing the *ApaI* restriction sites (rs7975232), restriction fragment length polymorphism analysis and gel electrophoresis.

Using the kit (Thermo Scientifc #K0781), DNA extraction was done followed by PCR using the primers.

F 5'CAGAGCATGGACAGGGAGCAA3'

R 5'GCAACTCCTCATGGCTGAGGTCTC3'.

The 20 µL PCR reaction mixture contained 3 µL 25 mM $MgCl_2$, 2 µL 10X NH_4SO_4 buffer, 3 µL 2.5 mM dNTPs, 0.5 µL 5U/µL Taq polymerase, 5 µL DNA, 1.5 µL 10 µM forward primer, 1.5 µL 10 µM reverse primer, and 3.5 µL water. For the amplification of the DNA sequence containing the *ApaI* restriction site (rs7975232), PCR underwent 35 cycles, each consisted of initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, annealing at 65°C for 45 seconds, extension at 72°C for 45 seconds, and then final extension at 72°C for 10 minutes. The PCR product was of 744 base pairs.

Restriction fragment length polymorphism (RFLP) analysis for *ApaI* was done using the enzyme Thermo Scientific *ApaI* (#ER1411). The 30 µL mixture contained 10 µL PCR product (0.1-0.5 µg), 2 µL 10X buffer B, 2 µL *ApaI* enzyme, and 16 µL nuclease free water. The mixture was incubated at 37°C for 8-16 h. Samples were run on 2% agarose gel and visualization was done under ultraviolet light and stored in the documentation system.

Statistical analysis

Data was analyzed using SPSS 24. T-test (for normally distributed data) and Mann-Whitney test (for not-normally distributed data) were used to compare age, BMI, hemoglobin, liver function tests, platelet count, prothrombin time and INR in cirrhotic and non-cirrhotic patients. Frequencies of *ApaI* polymorphisms were studied in accord with the Hardy-Weinberg equilibrium. Chi-square test was used to assess the association of vitamin D polymorphisms with the response to treatment and with cirrhosis. Logistic regression was used to find out the association of *ApaI* and other independent variables with the response to treatment. A p-value of < 0.05 was considered significant.

RESULTS

There were two groups, responders and non-responders, with 66 patients in each. There were 40 (63.6%) males and 26 (36.4%) females in each group and 33 (50%) cirrhotic and 33 (50%) non-cirrhotic patients in each group.

The restriction fragment length polymorphism analysis showed a single band of 744 base pairs in the AA wild homozygous genotype, three bands of 744, 527, and 217 base pairs in the Aa heterozygous genotype, and two bands of 527 and 217 base pairs in the aa homozygous mutant genotype (Fig. 1).

The frequencies of the AA, Aa, and aa *ApaI* genotypes were 28 (42.4%), 27 (40.9%), and 11 (16.7%) in responders and 22 (33.3%), 26 (39.4%), and 18 (27.3%) in non-responders. The allelic distribution for the "A" and "a" alleles was 83 (62.9%) and 49 (37.1%) in responders and 70 (53%) and 62 (47%) in non-responders. Logistic regression showed *ApaI* genotype ''aa'' as a significant predictor of treatment failure (p-value= .024, OR= 3.589, 95% CI= 1.181-10.911) (Table I).

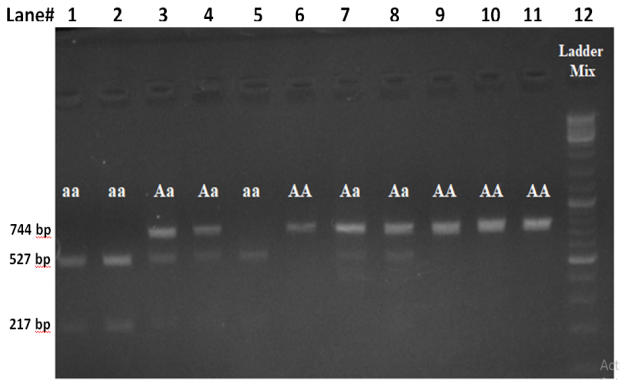

Fig. 1. RFLP pattern of *ApaI* VDR polymorphism (rs7975232) in 2% agarose gel showing homozygous wild AA genotype with a band of 744 bp (Lanes# 6, 9, 10, and 11), heterozygous Aa genotype with the bands 744, 527, and 217 bp (Lanes# 3, 4, 7, and 8), and homozygous mutant aa genotypes with the bands 527 and 217 bp (Lanes# 1, 2, and 5).

Table I. Logistic regression of potential predictors of response to treatment in chronic hepatitis C genotype 3 patients.

Variables	B	p-value	OR (95% CI)
AA	Referent		
aa	1.278	0.024*	3.589 (1.181-10.911)
Aa	0.698	0.141	2.009 (0.794-5.082)
Age	-0.032	0.238	0.969 (0.919-1.021)
Gender	0.200	0.635	1.221 (0.535-2.790)
Smoking	-0.572	0.276	0.564 (0.202-1.579)
BMI	0.013	0.672	1.013 (0.955-1.074)
Cirrhosis	0.030	0.961	1.030 (0.309-3.433)
Hemoglobin	-0.179	0.085	0.836 (0.682-1.025)
Platelets count	-0.003	0.223	0.997 (0.991-1.002)
PT	-0.509	0.091	0.601 (0.333-1.085)
INR	0.897	0.602	2.453 (0.084-71.383)
Total bilirubin	-0.211	0.825	0.810 (0.125-5.240)
Direct bilirubin	0.288	0.857	1.334 (0.058-30.832)
AST	0.004	0.789	1.004 (0.976-1.032)
ALT	0.009	0.420	1.009 (0.988-1.030)
ALP	0.003	0.756	1.003 (0.983-1.024)
Serum albumin	-0.0404	0.336	0.668 (0.293-1.521)

* p-value < .05 is significant. OR, Odd's ratio; CI, Confidence interval; BMI, Body mass index; PT, Prothrombin time; INR, International Normalized Ratio; AST, Aspartate aminotransferase; ALT, Alanine Aminotransferase; ALP, Alkaline phosphatase.

There was no association of *ApaI* VDR polymorphism with cirrhosis in responders and non-responders (Table II). The frequencies of *ApaI* VDR genotypes in males and females are shown in Table III and no significant difference was seen in the frequencies of *ApaI* VDR genotypes between males and females in both responder and non-responders

Table II. Association of *ApaI* VDR polymorphism with cirrhosis.

VDR genotypes	Responders[a] (n=66)		Non-Responders[b] (n=66)	
	Cirrhotic (n=33)	Non-cirrhotic (n=33)	Cirrhotic (n=33)	Non-cirrhotic (n=33)
AA	15	13	9	13
aa	5	6	10	8
Aa	13	14	14	12
Chi square test	0.271		1.103	
p-value	0.873		0.576	

* p-value < .05 is significant. [a] Responders are patients who achieved sustained virologic response (SVR) three months after completing the treatment (HCV-RNA negative). [b] Non-Responders are patients who did not achieve sustained virologic response (SVR) three months after completing the treatment (HCV-RNA positive).

Table III. Distribution of *ApaI* VDR genotypes by gender.

ApaI genotype	Responders[a] (n=66)		Non-Responders[b] (n=66)	
	Male n (%)	Female n (%)	Male n (%)	Female n (%)
AA	18 (45%)	10 (38.5%)	10 (25%)	12(46.2%)
aa	6 (15%)	5 (19.2%)	12 (30%)	6 (23.1%)
Aa	16 (40%)	11 (42.3%)	18 (45%)	8 (30.8%)
Total	40 (100%)	26 (100%)	40 (100%)	26 (100%)
Chi-Square test	0.349		3.202	
p-value	0.840		0.202	

* p-value < .05 is significant. For details of responders and non-responders, see Table II.

Significant differences were found in the levels of total bilirubin, direct bilirubin, ALP, serum albumin, PT, and INR between cirrhotic and non-cirrhotic patients in both the groups, responders and non-responders, and in the AST levels between cirrhotic and non-cirrhotic patients in the non-responder group (p-value < .05) (Tables IV and V).

Table IV. Age, BMI, platelets count, hemoglobin, LFTs, PT, and INR in cirrhotic and non-cirrhotic patients (in responders).

Variable	Mean±SD[a] / Mean rank[b]		t[a] / U[b]	p-value
	Cirrhotic (n=33)	Non-cirrhotic (n=33)		
Age (yrs)	32.92[b]	34.08[b]	525.5[b]	0.807
BMI (kg/m^2)	26.84±7.21[a]	27.09±6.51[a]	-0.147[a]	0.883
Platelets(×10^3/µL)	35.11[b]	31.89[b]	491.5[b]	0.495
Hb (g/dL)	12.83±2.03[a]	13.46±1.89[a]	-1.313[a]	0.194
Total bilirubin (mg/dL)	43.58[b]	23.42[b]	212.0[b]	0.000*
Direct bilirubin (mg/dL)	44.71[b]	22.29[b]	174.5[b]	0.000*
AST (U/L)	37.68[b]	29.32[b]	406.5[b]	0.076
ALT (U/L)	64.79±23.73[a]	74±19.52[a]	-1.734[a]	0.088
ALP (IU/L)	114.82±24.33[a]	101.52±16.07[a]	2.621[a]	0.011*
Serum albumin (g/dL)	24.67[b]	42.33[b]	253.0[b]	.000*
PT (seconds)	41.17[b]	25.83[b]	291.5[b]	0.001*
INR	45.91[b]	20.09[b]	102.0[b]	.000*

* p-value < .05 is significant. [a]T-test was used; [b]Mann-Whitney test was used. For other abbreviations, see Table I.

Table V. Age, BMI, platelets count, hemoglobin, LFTs, PT, and INR in cirrhotic and non-cirrhotic patients (in non-responders).

Variable	Mean±SD[a] / Mean Rank[b]		t[a] / U[b]	p-value
	Cirrhotic (n=33)	Non-cirrhotic (n=33)		
Age (yrs)	49.18±87.43[a]	48.85±7.75[a]	0.178[a]	0.859
BMI (kg/m^2)	35.70[b]	31.30[b]	472.0[b]	0.352
Platelets (×10^3/µL)	37.59[b]	29.41[b]	409.5[b]	0.082
Hb (g/dL)	12.69±2.07[a]	12.79±1.88[a]	-0.199[a]	0.843
Total bilirubin (mg/dL)	41.36[b]	25.64[b]	285.0[b]	0.001*
Direct bilirubin (mg/dL)	42.88[b]	24.12[b]	235.0[b]	0.000*
AST (U/L)	35.97[b]	31.03[b]	463.0[b]	0.295
ALT (U/L)	64.85±23.43[a]	77.06±19.40[a]	-2.306[a]	0.024*
ALP (IU/L)	115.15±23.25[a]	104.48±15.91[a]	2.175[a]	0.033*
Serum albumin (g/dL)	26.35[b]	40.65[b]	308.5[b]	0.002*
PT (seconds)	42.73[b]	24.27[b]	240.0[b]	.000*
INR	46.36[b]	20.64[b]	120.0[b]	.000*

For statistical details and abbreviations see Tables I and IV.

DISCUSSION

Our study found the mutant homogenous *ApaI* genotype aa to be a risk factor and a predictor of treatment failure (OR= 3.589, 95% CI= 1.181-10.911). The mutant homogenous *ApaI* aa genotype was also found to be a risk factor for treatment failure by Baur *et al*. (2012) (OR=2.67, 95% CI= 1.24-5.70). They found *ApaI* polymorphism to be associated with failure to pegylated interferon and ribavirin treatment.

Contrary to our findings, Thanapirom *et al*. (2019) found no association between *ApaI* polymorphism and the response to treatment; however, this could have been due to the presence of HCV genotypes 1, 2, 3, and 4 patients

in their study whereas our study included only genotype 3 hepatitis C patients and the hepatitis C genotype could influence the response to treatment and might interact with the pharmacokinetics of the different drugs.

Wang *et al*. (2016) found no association between *ApaI* VDR polymorphism and the response to treatment in HCV Chinese patients and similar results were reported by Abdelsalam *et al*. (2016) in genotype 4 Egyptian patients. In all these studies, the used antiviral treatment was interferon and ribavirin whereas in our study the treatment was directly acting antiviral treatment. The difference in the results could be due to pharmacogenetics, ethnicity, or the different HCV genotypes as the three factors do affect the response to treatment.

The association of *ApaI* polymorphism with failure to treatment (daclatasvir and sofosbuvir with ribavirin in cirrhotic patients) could be explained in view of the effect of *ApaI* polymorphism in forming a VDR protein that is less active and might cause a disturbance in the balance of T helper cell type 1/ T helper cell type 2, thereby causing diminished activity of the signaling pathways of vitamin D (Triantos *et al*., 2018).

This study is the first on the association of *ApaI* polymorphisms with the response to directly acting antiviral treatment i.e., daclatasvir and sofosbuvir (with ribavirin in cirrhotic patient) in chronic hepatitis C genotype 3 patients. The previous studies were conducted on patients who received pegylated interferon and ribavirin and none was specific to hepatitis C genotype 3 patients. *ApaI* VDR polymorphism could be considered as a new marker to predict the failure of treatment in chronic hepatitis C patients.

CONCLUSION

ApaI VDR genotype aa is a predictor of failure to treatment in chronic hepatitis C genotype 3 patients and can be considered as a risk factor for treatment failure.

Conflict of interest

The authors have declared no conflict of interest.

Funding disclosure

None to declare

Disclaimer

None to declare.

REFERENCES

Abbas, Z. and Abbas., M., 2020. The cost of eliminating hepatitis C in Pakistan. *Lancet Glob. Hlth.*, **8**: e323-e324. https://doi.org/10.1016/S2214-109X(20)30036-X

Abdelsalam, A., Rashed, L., Salman, T., Hammad, L. and Sabry, D., 2016. Molecular assessment of vitamin D receptor polymorphism as a valid predictor to the response of interferon/ ribavirin-based therapy in Egyptian patients with chronic hepatitis C. *J. Dig. Dis.*, **17**: 547-553. https://doi.org/10.1111/1751-2980.12353

Adams, J.S. and Hewison, M., 2008. Unexpected actions of vitamin D: New perspectives on the regulation of innate and adaptive immunity. *Nat. Clin. Pract. Endocrinol. Metab.*, **4**: 80-90. https://doi.org/10.1038/ncpendmet0716

Al-Aqmer, N.H.H., Zulfiqar, S., Zulfiqar, S., Shakoori, A.R. and Izhar, M., 2021. FokI vitamin D receptor polymorphism as a predictor of response to hepatitis C antiviral treatment. *Pak. J. Physiol.*, **17**: 3-6. Epub: http://www.pps.org.pk/PJP/17-3/Noora.pdf

Al Kanaani, Z., Mahmud, S., Kouyoumjian, S.P. and Abu-Raddad, L.J., 2018. The epidemiology of hepatitis C virus in Pakistan: Systematic review and meta-analyses. *R. Soc. Open Sci.*, **5**: 180257. https://doi.org/10.1098/rsos.180257

Altaf, A. and Pasha, S.K., 2020. Hepatitis C elimination in Pakistan is a distant dream unless government controls the health sector. *J. Pak. med. Assoc.*, **70**: 2454-2456. https://doi.org/10.47391/JPMA.384

Arai, T., Atsukawa, M., Tsubota, A., Kondo, C., Shimada, N., Abe, H., Itokawa, N., Nakagawa, A., Okubo, T., Aizawa, Y. and Iwakiri, K., 2015. Vitamin D-related gene polymorphisms do not influence the outcome and serum vitamin D level in pegylated interferon/ribavirin therapy combined with protease inhibitor for patients with genotype 1b chronic hepatitis C. *J. Med. Virol.*, **87**: 1904-1912. https://doi.org/10.1002/jmv.24244

Baur, K., Mertens, J.C., Schmitt, J., Iwata, R., Stieger, B., Frei, P., Seifert, B., Bischoff Ferrari, H.A., von Eckardstein, A., Müllhaupt, B. and Geier, A., 2012. The vitamin D receptor gene bAt (CCA) haplotype impairs the response to pegylated-interferon/ ribavirin-based therapy in chronic hepatitis C patients. *Antivir. Ther.*, **17**: 541-547. https://doi.org/10.3851/IMP2018

García-Martín, E., Agúndez, J.A., Maestro, M.L., Suárez, A., Vidaurreta, M., Martínez, C., Fernández-Pérez, C., Ortega, L. and Ladero, J.M., 2013. Influence of vitamin D-related gene polymorphisms (CYP27B and VDR) on the response to interferon/ribavirin therapy in chronic hepatitis C. *PLoS One*, **8**: e74764.

https://doi.org/10.1371/journal.pone.0074764

Gower, E., Estes, C., Blach, S., Razavi-Shearer, K. and Razavi, H., 2014. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J. Hepatol.*, **61**: S45-S57. https://doi.org/10.1016/j.jhep.2014.07.027

Haqqi, A., Munir, R., Khalid, M., Khurram, M., Zaid, M., Ali, M., Shah, Z.H., Ahmed, H. and Afzal, M.S., 2019. Prevalence of hepatitis C virus genotypes in Pakistan: current scenario and review of literature. *Viral Immunol.*, **32**: 402-413. https://doi.org/10.1089/vim.2019.0058

Hill, A.M., Nath, S. and Simmons, B., 2017. The road to elimination of hepatitis C: Analysis of cures versus new infections in 91 countries. *J. Virus Erad.*, **3**: 117-123. PMID: 28758018. https://doi.org/10.1016/S2055-6640(20)30329-0

Mahmud, S., Al Kanaani, Z. and Abu-Raddad, L.J., 2019. Characterization of the hepatitis C virus epidemic in Pakistan. *BMC Infect. Dis.*, **19**: 809. https://doi.org/10.1186/s12879-019-4403-7

Perz, J.F., Armstrong, G.L., Farrington, L.A., Hutin, Y.J. and Bell, B.P., 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J. Hepatol.*, **45**: 529-538. https://doi.org/10.1016/j.jhep.2006.05.013

Pike, J.W. and Meyer, M.B., 2012. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D3. *Rheum. Dis. Clin. N. Am.*, **38**: 13-27. https://doi.org/10.1016/j.rdc.2012.03.004

Polaris Observatory HCV Collaborators, 2017. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol. Hepatol.*, **2**: 161-176. https://doi.org/10.1016/S2468-1253(16)30181-9

Stanaway, J.D., Flaxman, A.D., Naghavi, M., Fitzmaurice, C., Vos, T., Abubakar, I., Abu-Raddad, L.J., Assadi, R., Bhala, N., Cowie, B., Forouzanfour, M.H., Groeger, J., Hanafiah, K.M., Jacobsen, K.H., James, S.L., MacLachlan, J., Malekzadeh, R., Martin, N.K., Mokdad, A.A., Mokdad, A.H., Murray C.J.L., Plass, D., Rana, S., Rein, D.B., Richardus, J.H., Sanabria, J., Saylan, M., Shahraz, S., So, S., Vlassov, V.V., Weiderpass, E., Wiersma, S.T., Younis, M., Yu, C., El Sayed Zaki, M. and Cooke, G.S., 2016. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet,* **388**: 1081-1088. https://doi.org/10.1016/S0140-6736(16)30579-7

Thanapirom, K., Suksawatamnuay, S., Sukeepaisarnjaroen, W., Tangkijvanich, P., Thaimai, P., Wasitthankasem, R., Poovorawan, Y. and Komolmit, P., 2019. Genetic associations of vitamin D receptor polymorphisms with advanced liver fibrosis and response to pegylated interferon-based therapy in chronic hepatitis C. *PeerJ.*, **7**: e7666. https://doi.org/10.7717/peerj.7666

Triantos, C., Aggeletopoulou, I., Kalafateli, M., Spantidea, P.I., Vourli, G., Diamantopoulou, G., Tapratzi, D., Michalaki, M., Manolakopoulos, S., Gogos, C., Kyriazopoulou, V., Mouzaki, A. and Thomopoulos, K., 2018. Prognostic significance of vitamin D receptor (VDR) gene polymorphisms in liver cirrhosis. *Sci. Rep.*, **8**: 14065. https://doi.org/10.1038/s41598-018-32482-3

Umer, M. and Iqbal, M., 2016. Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. *World J. Gastroenterol.*, **22**: 1684-1700. https://doi.org/10.3748/wjg.v22.i4.1684

Wang, C., Wu, Q., Wei, X.T., Li, Z.Z. and Lei, X.Z., 2016. Association of vitamin D receptor polymorphisms with response to antiviral therapy in patients with chronic hepatitis C. *Sichuan Da Xue Xue Bao Yi Xue Ban.*, **47**: 227-231.

Wu, M., Yue, M., Huang, P., Zhang, Y., Xie, C., Yu, R., Li, J. and Wang, J., 2016. Vitamin D level and vitamin D receptor genetic variations contribute to HCV infection susceptibility and chronicity in a Chinese population. *Infect. Genet. Evol.*, **41**: 146-152. https://doi.org/10.1016/j.meegid.2016.03.032

Younossi, Z., Park, H., Henry, L., Adeyemi, A., Stepanova, M., 2016. Extrahepatic manifestations of hepatitis C: A meta-analysis of prevalence, quality of life, and economic burden. *Gastroenterology*, **150**: 1599-1608. https://doi.org/10.1053/j.gastro.2016.02.039