Original Article

# Association between Hepatitis C Virus Viremia and the rs12979860, rs2228145 and rs1800795 SNP (CT/AC/GG) Genotype in Saudi Kidney Transplant Recipients

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## Abstract

**Background:** Hepatitis C virus (HCV) is a major health problem, particularly in high-risk groups such as kidney transplant recipients, where it can adversely affect graft survival and increase the relative risk for mortality. Recently, the role of genetic variation among HCV patients in determining the outcome of infections has been under investigation.

**Objective:** To investigate the association of single-nucleotide polymorphisms (SNPs) rs12979860 (located within the interleukin-28B locus), rs2228145 (interleukin-6 receptor) and rs1800795 (interleukin-6 promoter) with HCV viremia in renal transplant patients.

**Materials and Methods:** In this analytical cross-sectional study, 149 kidney transplant recipients, 82 males (median age: 41 years) and 67 females (median age: 45 years), were screened for HCV RNA in blood using real-time polymerase chain reaction and genotyped by sequencing (rs12979860) and restriction fragment length polymorphism (rs2228145 and rs1800795).

**Results:** HCV RNA was detected in 17 (11.41%) of the 149 patients. There was no statistically significant association between the studied SNPs and HCV viremia. However, a combination of the CT/AC/GG genotype was significantly associated with HCV viremia (odds ratio: 5.4). The genotype AA of rs2228145 in the IL-6 receptor was associated with viremia levels of $>10^5$ copies/ml (odds ratio: 5.96).

**Conclusion:** To the best of the authors' knowledge, this is the first study that has shown that the CT/AC/GG genotype has an impact on HCV viremia in kidney transplant patients. Therefore, such SNP genotypes may potentially be used to identify transplant patients at risk of HCV infection.

**Keywords:** Hepatitis C virus, interleukin-28B, interleukin-6, kidney transplant, Saudi Arabia

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## INTRODUCTION

Hepatitis C virus (HCV) is a major health problem, as it has infected an estimated 150 million people worldwide.[1,2] Despite the fact that the primary infection with HCV is associated with mild symptoms or is asymptomatic in some cases, it is cleared in only about 30% of the infected individuals.[3] Among those who develop chronic hepatitis, the most common complications are fibrosis (20%–30%) and cirrhosis (10%–20%), of whom 1%–4% develop hepatocellular carcinoma.[4]

It is important to understand the factors that lead to spontaneous recovery, as this may provide vital insight into the pathogenesis of hepatitis C. Several viral and host factors including the patient's age, gender, genetic makeup, body weight, hepatic steatosis, alcohol consumption and coinfection with other viruses including hepatitis B virus and HIV have been reported to determine the outcome of HCV infection. The adaptive cellular immune response to HCV infection seems to play a major role in the elimination of acute HCV infection.[5] However, certain factors such as an inefficient T-cell response, which fails to completely clear HCV from the liver due to the appearance of rapid HCV escape mutants and other factors related to T-cell response, have been shown to be responsible for the chronicity of HCV infection.[6,7]

Studies have shown that genetic variation in HCV patients plays a role in determining the outcome of infections. A study found that response to the standard combination of treatment with pegylated interferon and ribavirin in infection with certain HCV genotypes varies from no response to partial response or relapse, depending on genetic factors.[8] Specifically, the most commonly described single-nucleotide polymorphism (SNP) associated with the outcome of HCV therapy is rs12979860, which is located near the interleukin (IL)-28B gene.[9,10] The CC genotype of rs12979860 was found to be associated with better response to treatment in Europeans, Hispanics and African Americans compared with the non-CC genotypes.[9] The homozygous G allele of rs8099917 has been shown to be significantly associated with lack of response to treatment in Japanese, Australian and European patients with HCV genotype 1 infection.[11,12] These two SNPs, among others located in the same region, have been also linked to the rate of expression of interferon genes, duration of treatment and chronicity of infection and its complications such as fibrosis.[8,13] Several other SNPs located in the ribavirin transporters, vitamin D receptor and IL-6 gene have also been found to affect the outcome of HCV infection.[14-16] HCV is a major health problem, particularly for high-risk groups such as kidney transplant recipients, as HCV adversely affects graft survival and increases the relative risk for mortality.[17] Furthermore, the risk of new-onset diabetes after transplantation was found to be markedly higher in HCV-positive patients, probably due to inhibition of the insulin regulatory pathways by the virus.[18]

To the best of the authors' knowledge, one study from Saudi Arabia has investigated the role of IL-28B polymorphism in the virological response to standard therapy in patients with chronic HCV infection, while another study found that rs8099917-GG genotype is associated with increased serum aIL-28B levels in HCV patients; however, both studies did not include kidney transplant recipients.[19,20] The aim of this work was to assess the rate of HCV viremia in renal transplant recipients, as a risk group, using real-time polymerase chain reaction (PCR) and evaluate its relation with rs12979860 (IL-28B). In addition, high levels of serum IL-6 were previously found to be associated with HCV treatment failures.[21,22] The rs1800795 polymorphism in IL-6 promoter was found to affect IL-6 expression.[23] Similarly, the SNP rs2228145 in the IL-6 receptor was found to be associated with increased soluble IL-6 receptor levels, which might influence IL-6 signaling and functions.[24,25] Hence, we also investigated the genetic polymorphism of these two SNPs in the Saudi kidney transplant recipients and their relation to HCV viremia.

## MATERIALS AND METHODS

### Study population

In this analytical cross-sectional study, a group of 149 patients who received a renal allograft and/or attended follow-up care at the nephrology clinics of King Fahd Military Medical Complex, Dhahran, Saudi Arabia, from January to April 2009 were included.[26] The blood samples were collected and stored at −20°C until DNA/RNA extraction. The cohort comprised 82 males (55%; median age: 41 years) and 67 females (45%; median age: 45 years). All patients were Saudis.

Hypertension, glomerulonephritis and diabetes mellitus were the most frequent causes of kidney failure in this group (19.7%, 19.7% and 13.2%, respectively), whereas in 25% of the cases, the cause was unknown. More than half of the patients (51.3%) underwent kidney transplantation in Saudi Arabia, whereas the remaining underwent the procedures in Pakistan, the Philippines, Egypt, India, Iran, Lebanon and China. All patients were receiving a triple immunosuppressive regimen comprising cyclosporine, prednisolone and either azathioprine or mycophenolate

mofetil.[26] No antiviral drug was given to these patients during the period of the study. Informed consent was obtained from all participants before enrollment in the study.

### Detection of hepatitis C virus RNA in plasma

RNA was extracted from plasma samples with Ribo-Virus RNA/DNA extraction kit according to the manufacturer's instructions (Sacace Biotechnologies, Como, Italy). The samples were spiked with an internal control to monitor both RNA extraction and amplification detection. For HCV RNA detection and quantification, the HCV Real-TM Quant kit was used according to the manufacturer's instructions (Sacace Biotechnologies, Como, Italy). The PCR reaction was run on the smart cycler (Cepheid, California, USA).

### Genotyping

DNA extraction was performed on 200 μl of blood using blood and tissue kit (Qiagen, Hilden, Germany). DNA fragment of 200 bp containing the rs12979860 SNP was amplified by PCR using the following primers: rs860For (5'-TCTTCCTCCTGCGGGACAAG-3') and rs860Rev (5'-GGAGCGCGGAGTGCAATTCAAC-3'). The amplicon was run on 1.5% agarose gel, and then, the band of the expected size was excised and purified with gel purification kit (Qiagen, Hilden, Germany). The purified DNA was sequenced in both forward and reverse directions using BigDye terminator mix on the 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). Genotyping of the SNP G-174C (rs1800759) in the IL-6 promoter and SNP D358A (rs8192284) in the IL-6 receptor was performed by PCR-restriction fragment length polymorphism, as described previously.[27]

### Statistical analysis

The Haplotype Analysis software was used to calculate the frequency of genotype groups (Forest Genetics and Forest Tree Breeding, Georg-August University Goettingen, Germany) (http://www.uni-goettingen.de/en/134935.html).[28] The Haploview software (Daly Lab at the Broad Institute, Cambridge, MA 0214, USA) (https://www.broadinstitute.org/haploview/haploview) was used to study the linkage disequilibrium between the SNPs. Frequency tables and statistical association calculations were performed using SPSS version 25 (SPSS UK, Woking, UK) and OpenEpi version 7.2.2.6 (CDC, Atlanta, Georgia, USA).

### Ethical approvals

This study was approved by the Ethical Committee of Biomedical Research at King Fahd Military Medical Complex and also by the Research Ethics Committee at Imam Abdulrahman Bin Faisal University (number 2011014, January 10, 2011).

## RESULTS

HCV RNA was detected in 17 of the 149 (11.41%) patients, of which 10 were female [Table 1]. All other clinical data of the HCV-positive patients are listed in Table 1. No statistically significant difference was observed between gender and HCV viremia (41% males and 59% females). The HCV RNA viral load was $<10^5$ copies/ml in 13 patients (mean: $3.2 \pm 2.6 \times 10^4$ copies/ml; range $1.35–7.9 \times 10^4$) and $> 10^5$ copies/ml (12.23, 4.47, 3.14 and $2.97 \times 10^5$ copies/ml) in the other 4 patients.

None of the studied SNP genotypes were found to have a statistically significant association with the presence of HCV RNA [Table 2]. However, a comparison of

**Table 1: Data of the hepatitis C virus-positive kidney transplant recipients**

| Sex | Age years | Blood group | Period since transplantation date (years) | Transplantation place | Donor | Cause of renal failure | HCV viral load copies/ml |
|---|---|---|---|---|---|---|---|
| Male | 47 | O+ | 1.6 | KSA | Live | Glomerulonephritis | 13,513 |
| Female | 23 | O+ | 6.6 | Iran | Live | SLE | 13,601 |
| Female | 54 | B+ | 1.8 | KSA | Live | Glomerulonephritis | 13,945 |
| Female | 34 | B+ | 3.9 | KSA | Live | Unknown | 14,021 |
| Female | 55 | O+ | 4.3 | Egypt | Live | Diabetic nephropathy | 14,233 |
| Male | 69 | O− | 7.7 | The Philippines | Live | Unknown | 15,123 |
| Female | 48 | O+ | 2.3 | Pakistan | Live | Glomerulonephritis | 15,700 |
| Female | 32 | O+ | 8.7 | The Philippines | Live | Renal stone | 15,700 |
| Male | 43 | A+ | 15 | KSA | Live | Hypertension | 18,910 |
| Male | 30 | O+ | 4.7 | KSA | Cadaver | Alport syndrome | 45,990 |
| Male | 43 | O+ | 6.9 | Pakistan | Live | Unknown | 78,553 |
| Female | 42 | O+ | 7.2 | Egypt | Live | Glomerulonephritis | 78,910 |
| Male | 35 | O+ | 3.8 | Pakistan | Live | Hypertension | 79,000 |
| Female | 36 | AB+ | 11 | KSA | Live | Unknown | 297,128 |
| Female | 53 | AB+ | 5.7 | KSA | Cadaver | Hypertension | 314,023 |
| Female | 52 | O+ | 7.5 | KSA | Live | Chronic pyelonephritis | 447,232 |
| Male | 39 | O+ | 1.6 | The Philippines | Live | Diabetic nephropathy | 1,223,049 |

HCV – Hepatitis C virus; SLE – Systemic lupus erythematosus; KSA – Kingdom of Saudi Arabia

**Table 2: Frequency of single-nucleotide polymorphism genotypes with hepatitis C virus infection**

| Genotypes | HCV RNA | | Total, *n* (%) | OR (95% CI) |
|---|---|---|---|---|
| | Negative, *n* (%) | Positive, *n* (%) | | |
| rs12979860 (IL-28B) | | | | |
| CC | 58 (89.2) | 7 (10.8) | 65 (43.6) | 0.89 (0.29–2.75) |
| CT | 59 (86.8) | 9 (13.2) | 68 (45.6) | 1.39 (0.46–4.27) |
| TT | 15 (93.8) | 1 (16.2) | 16 (10.8) | 0.49 (0.02–3.96) |
| rs2228145 (IL-6 receptor) | | | | |
| AA | 67 (90.5) | 7 (9.5) | 74 (49.7) | 0.68 (0.22–2.09) |
| AC | 46 (86.8) | 7 (13.2) | 53 (35.6) | 1.31 (0.42–4.06) |
| CC | 19 (86.4) | 3 (13.6) | 22 (14.7) | 1.27 (0.26–5.41) |
| rs1800795 (IL-6 promoter) | | | | |
| GG | 97 (88.2) | 13 (11.8) | 110 (73.8) | 1.17 (0.33–4.59) |
| GC | 32 (88.9) | 4 (11.1) | 36 (24.2) | 0.96 (0.24–3.49) |
| CC | 3 (100) | 0 | 3 (2.0) | 0.00 (0.0–15.87) |

HCV – Hepatitis C virus; IL – Interleukin; OR – Odds ratio; CI – Confidence interval

all genotype groups showed that the genotype groups CT/AC/GG (IL-28B/IL-6R/IL-6P) had a statistically significant higher rate of HCV viremia than other genotype groups (odds ratio [OR]: 5.4) [Table 3].

The AA genotype of rs2228145 in the IL-6 receptor was associated with HCV viral load >$10^5$ copies/ml (OR: 5.96) [Table 4]; however, this association was statistically nonsignificant.

## DISCUSSION

The current study measured the percentage of HCV infections among kidney transplant recipients. It is unclear when the HCV-positive patients contracted the infection, as no data on the history of HCV infection were available. However, the infection may not have been acquired from the transplanted organ itself, as testing for HCV is a well-established laboratory assay and routinely performed for organ donors. The authors assume that the patients most likely acquired the infection, conceivably, due to the long-term hemodialysis that usually precedes or accompanies renal transplantation. The prevalence of HCV infection in hemodialysis patients in various parts of the world varies between 10% and 70%, in addition to the transplantation itself, which can also be a cause of HCV transmission.[29-31] The prevalence of HCV in Saudi Arabia is relatively low in blood donors.[32] However, this percentage increases among risk groups such as drug users, patients on dialysis and renal transplant recipients, as estimated by anti-HCV antibodies.[33-35]

A significantly higher rate of viremia was detected in patients with a combination of the CT/AC/GG genotype (IL-28B/IL-6R/IL-6P) than other patients. This suggests that HCV efficiently establishes infection in patients with these variants. This genotype combination was found in 12.1% of the study population, of which one-third were infected with HCV. However, we cannot rule out the presence of other contributing genetic factors outside the studied loci.

**Table 3: Association of genotype groups with hepatitis C virus infection**

| IL-28B/ IL-6R/IL-6P | HCV RNA | | Total, *n* (%) | OR (95% CI) |
|---|---|---|---|---|
| | Negative, *n* (%) | Positive, *n* (%) | | |
| CC/AA/GG | 21 (84) | 4 (16) | 25 (16.8) | 0.6 (0.18–2.38) |
| CC/AA/GC | 5 (83.3) | 1 (16.7) | 6 (4) | 0.6 (0.08–15.8) |
| CC/AC/GG | 16 (94.1) | 1 (5.9) | 17 (11.4) | 2.2 (0.35–49.5) |
| CC/AC/GC | 7 (100) | 0 | 7 (4.7) | Undefined |
| CC/CC/GG | 8 (88.9) | 1 (1.1) | 9 (6) | 1.0 (0.15–24.5) |
| CC/CC/GC | 1 (100) | 0 | 1 (0.7) | Undefined |
| CT/AA/GG | 24 (96) | 1 (4) | 25 (16.8) | 3.5 (0.59–78.3) |
| CT/AA/GC | 7 (87.5) | 1 (12.5) | 8 (5.4) | 0.9 (0.13–21.6) |
| CT/AC/GG | 12 (66.7) | 6 (33.3) | 18 (12.1) | 5.4 (1.58–17.3) |
| CT/AC/GC | 4 (100) | 0 | 4 (2.7) | Undefined |
| CT/AC/CC | 2 (100) | 0 | 2 (1.3) | Undefined |
| CT/CC/GG | 4 (100) | 0 | 4 (2.7) | Undefined |
| CT/CC/GC | 6 (85.7) | 1 (14.3) | 7 (4.7) | 0.8 (0.10–18.7) |
| TT/AA/GG | 9 (100) | 0 | 9 (6) | Undefined |
| TT/AA/CC | 1 (100) | 0 | 1 (0.7) | Undefined |
| TT/AC/GG | 2 (100) | 0 | 2 (1.3) | Undefined |
| TT/AC/GC | 2 (100) | 0 | 2 (1.3) | Undefined |
| TT/CC/GG | 1 (100) | 0 | 1 (0.7) | Undefined |
| TT/CC/GC | 0 | 1 (100) | 1 (0.7) | Undefined |
| Total | 132 (88.6) | 17 (11.4) | 149 | |

Twenty-seven possible genotypes could be generated of the combination of the two alleles at each of the three loci; however, only 19 genotypes were present in our study population. HCV – Hepatitis C virus; IL – Interleukin; OR – Odds ratio; CI – Confidence interval

In the current study, no significant association was found between any of the studied SNPs and HCV viremia. The high OR (5.96) between the AA genotype of SNP rs2228145 located in the IL-6 receptor and the levels of viremia of >$10^5$ copies/ml is suggestive of an association between these two parameters. However, this is not statistically significant because the confidence interval includes 1, which statistically nullifies a significant association. While the C allele of rs2228145 has been reported to protect against coronary heart disease and rheumatoid arthritis and to increase the risk of asthma,[36-38] to the best of the authors' knowledge, this is the first

**Table 4: Correlation between genotypes and hepatitis C virus viral load**

| Genotypes | HCV viral load | | Total, *n* (%) | OR (95% CI)* |
|---|---|---|---|---|
| | <105 IU/ml, *n* (%) | >105 IU/ml, *n* (%) | | |
| rs12979860 (IL-28B) | | | | |
| CC | 5 (71.4) | 2 (28.6) | 7 (41.2) | 1.74 (0.14–21.3) |
| CT | 7 (77.8) | 2 (22.2) | 9 (52.9) | 0.86 (0.07–10.5) |
| TT | 1 (100) | 0 | 1 (5.9) | 0 (0.0–61.75) |
| rs2228145 (IL-6 receptor) | | | | |
| AA | 4 (57.1) | 3 (42.9) | 7 (41.2) | 5.96 (0.48–195.4) |
| AC | 7 (100) | 0 | 7 (41.2) | 0 (0.0–1.38) |
| CC | 2 (66.7) | 1 (33.3) | 3 (17.6) | 1.76 (0.05–31.1) |
| rs1800795 (IL-6 promoter) | | | | |
| GG | 10 (76.9) | 3 (32.1) | 13 (76.5) | 0.9 (0.064–31.2) |
| GC | 3 (75.0) | 1 (25.0) | 4 (23.5) | 1.1 (0.03–15.5) |
| CC | 0 | 0 | 0 | – |

*Mid-*P* exact test. HCV – Hepatitis C virus; IL – Interleukin; OR – Odds ratio; CI – Confidence interval

report to show the association between rs2228145 and a viral infection. Ferreira *et al*.[25] found that the minor allele C of rs2228145 increases the level of soluble IL-6R and decreases the expression of the membrane-bound IL-6R on CD4+ cells and monocytes, leading to impaired IL-6 response. However, from the current study, it is unknown if the A allele provides a sustained IL-6 response owing to lack of information regarding the serum IL-6 levels in this study patients. Therefore, further studies are required to reveal any possible role the A allele may have in the persistence of HCV.

The rs12979860 CC genotype has previously been reported to strongly improve spontaneous and treatment-induced elimination of HCV infection.[39-41] A study from Saudi Arabia also found that the CC polymorphism in the IL-28B locus sustains response to treatment in patients infected with HCV genotype 4.[19] However, no such conclusion can be drawn in the current study because of the missing data regarding the treatment outcome of the study population. The current study did not find a statistically significant difference in the rate or level of viremia in the patients with any of the rs12979860 (IL-28B) variants. This is probably because of the triple immunosuppressive regimen that the patients had been receiving, rendering them unable to clear the virus. Therefore, the role of suppressed immune system in abrogating the role of CC variants cannot be excluded. Our results are in line with a study conducted in Egypt, where the CC genotype was not associated with HCV viral load.[42]

IL-6 is an inflammatory response marker that is considered to act as a mediator of innate immune response through receptor interaction with gp130, resulting in an increase in proinflammatory genes. IL-6 has also been demonstrated to induce the differentiation of T-helper 17 (Th-17) cells which, in turn, use IL-17 to enhance proinflammatory gene transcription.[43] A significantly higher level of IL-6 has been documented in patients with HCV than in healthy individuals.[22,44] When compared with successfully treated patients, previous studies have found higher levels of IL-6 in patients with treatment failure.[21,22] The majority of the viremic patients in the current study were of the GG variant in IL-6 promoter (rs1800795), with a higher proportion having a viremia level of $>10^5$ copies/ml, and the remaining were of GC genotype. The GG and GC genotypes of IL-6 promoter (rs1800795) have been reported to be high producers of IL-6.[23]

The small number of HCV-positive cases (17 cases) in this study weakens the power of statistical analysis. In addition, lack of data on the serum levels of IL-6 or IL-28B precludes conclusion on the effect of the SNPs on the IL-6 or IL-28B expression and its correlation to HCV. Therefore, more studies are required with larger HCV-positive population to explain the effect of these SNPs on HCV infection in kidney transplant recipients.

## CONCLUSION

This study found that none of the SNPs within the IL-28B locus, rs2228145 (IL-6 receptor) and rs1800795 (IL-6 promoter) individually have a significant impact on HCV infection in kidney transplant recipients. However, the CT/AC/GG genotype (IL-28B/IL-6R/IL-6P) may enhance establishment of HCV infection in these patients. Therefore, the authors suggest further studies with larger HCV-positive sample sizes to validate this finding, as it could help create a panel of HCV risk assessment indicators in kidney transplant recipients.

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## Ethical considerations

Ethical approval for this study was obtained from the Ethical Committee of Biomedical Research at King Fahd Military Medical Complex as well as from the Research Ethics Committee at Imam Abdulrahman Bin Faisal University (number 2011014, date January 10, 2011). Written informed consent was obtained from all participants before inclusion in this study.

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## Conflicts of interest

There are no conflicts of interest.

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