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(Article begins on next page)
CCR5-Δ32 and IL28B polymorphisms in treatment of HCV infection

**CCR5-Δ32 genotype does not improve predictive value of IL28B polymorphisms for treatment response in chronic HCV infection**

Vijayaprakash Suppiah¹,², Nicola J Armstrong³,⁴, Kate S. O'Connor², Thomas Berg⁵,⁶, Martin Weltman⁷, Maria Lorena Abate⁸, Ulrich Spengler⁹, Margaret Bassendine¹⁰, Gregory J. Dore¹¹,¹², William L. Irving¹³, Elizabeth Powell¹⁴,¹⁵, Jacob Nattermann⁹, Stephen Riordan¹⁶, Graeme J. Stewart², Jacob George¹, David R. Booth², Golo Ahlenstiel¹, for the International Hepatitis C Genetics Consortium (IHCGC).

¹ Storr Liver Unit, Westmead Millennium Institute, University of Sydney, Sydney, Australia, ² Institute for Immunology and Allergy Research, Westmead Millennium Institute, University of Sydney, Sydney, Australia, ³ Cancer Research Program, Garvan Institute for Medical Research and ⁴ School of Mathematics and Statistics, University of New South Wales, Sydney, Australia, ⁵ Medizinische Klinik m.S. Hepatologie und Gastroenterologie, Charité, Campus Virchow-Klinikum, Universitätsexmedizin Berlin, Germany, ⁶ Department of Hepatology, Clinic for Gastroenterology and Rheumatology, University Clinic Leipzig, Leipzig, Germany, ⁷ Department of Gastroenterology and Hepatology, Nepean Hospital, Sydney, Australia, ⁸ Liver Physiopathology Lab, Department of Internal Medicine, University of Turin, Turin, Italy, ⁹ Department of Internal Medicine I, University of Bonn, Bonn, Germany, ¹⁰ Liver Research Group, Institute of Cellular Medicine, Medical School, Newcastle University, Newcastle upon Tyne, United Kingdom, ¹¹ National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia, ¹² St Vincent's Hospital, Sydney, Australia, ¹³ NIHR Biomedical Research Unit in Gastroenterology and the Liver, University of Nottingham, Nottingham, United Kingdom, ¹⁴ Princess Alexandra Hospital, Department of Gastroenterology and Hepatology, Woolloongabba, ¹⁵ The University of Queensland, School of Medicine, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia.
CCR5-Δ32 and IL28B polymorphisms in treatment of HCV infection

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Address for Correspondence:

Professor Jacob George

Storr Liver Unit, Westmead Hospital

Hawkesbury Road, Westmead, NSW 2145, Australia

Tel.: +61 2 9845 7705

Fax: +61 2 9635 7582

jacob.george@sydney.edu.au

Abbreviations: ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.
Abstract

**Background:** IL28B polymorphisms strongly predict spontaneous and treatment-induced clearance of HCV infection. A recent study proposed a 32-base pair deletion in the CCR5 gene (CCR5-Δ32) interacting with the IL28B polymorphisms to influence spontaneous HCV clearance. The aim of this study was to clarify the role of CCR5-Δ32 in treatment-induced clearance of chronic hepatitis C (CHC).

**Methods:** A cross-sectional cohort of 758 Caucasian patients with CHC genotype 1 (341 responders and 417 non-responders) who had received standard of care dual therapy with IFN-α and ribavirin (RBV) were genotyped for the CCR5-Δ32 and IL28B polymorphisms to examine their interaction with respect to treatment response.

**Results:** CCR5-Δ32 did not influence treatment-induced recovery to IFN-α/RBV in CHC and did not improve prediction of SVR in the context of the IL28B polymorphisms in a multivariate model. CCR5-Δ32 homozygotes were significantly more frequent in those with CHC than healthy controls in the Europeans cohorts (2.9% vs 0.4%, p<0.0001), but not in Australians of European ancestry.

**Conclusion:** CCR5-Δ32 does not influence treatment response to IFN-α/RBV in the context of IL28B polymorphisms. Although CCR5-Δ32 may affect viral clearance within closely controlled geographic and genetic environments, we found no effect in larger cohorts treated with dual therapy.

**Key words:** CCR5, IL28B, HCV, SVR
INTRODUCTION

Hepatitis C virus (HCV) is a major cause of blood-borne hepatitis with an estimated worldwide prevalence of 3%\(^1\). Chronic infection causes hepatic inflammation eventually resulting in fibrosis and cirrhosis, and ultimately liver failure (reviewed in\(^2\)). Cytokines and chemokines are key players in this process through a complex network of interactions that regulate innate and adaptive immune responses to viral infection, and recruitment of inflammatory cells to the liver\(^3\). Importantly, lymphocytes infiltrating HCV-infected livers have been shown to express high levels of the CC-chemokines CCL3 (macrophagic inflammatory protein 1\(\alpha\), MIP-1\(\alpha\)), CCL4 (macrophagic inflammatory protein 1\(\beta\), MIP-1\(\beta\)), CCL5 (regulated upon activation, normal T cell expressed and secreted, RANTES) and their receptor, CC-chemokine receptor 5 (CCR5), suggesting a Th1-mediated antiviral response by the host to the virus\(^4\).

Several studies have linked HCV proteins to the expression of CCR5 ligands: Soo et al. showed that the expression of full-length HCV led to the induction of both CCR5 mRNA and protein in HeLa, Huh7 and HepG2 cell lines\(^5\). An increase in CCL5 levels by CD8\(^+\) T cells was reported to be related to the HCV E2 protein\(^6\). Finally, chronic HCV infection has been shown to lead to reduced surface expression of CCR5 on peripheral blood T cells\(^7\).

CCR5 has been previously identified as the major co-receptor for human immunodeficiency virus type 1 (HIV-1)\(^8\). A 32 bp deletion in exon 4 of CCR5 (CCR5-\(\Delta32\)) leads to truncation and loss of function of the receptor\(^9\). Homozygosity for this deletion confers high level (but not complete) resistance to HIV-1 infection, while heterozygotes show delayed progression to AIDS\(^10,\ 11\). An increased frequency of CCR5-\(\Delta32\) homozygosity was observed in HCV infected individuals compared to healthy controls (7.8% vs 1.0%)\(^12\). In a later Irish study, the authors found that CCR5-\(\Delta32\) was significantly associated with increased spontaneous
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clearance. Recently, Nattermann et al. have shown, in a very well defined patient cohort (n=396) infected with HCV genotype 1 from a single source, that CCR5-Δ32 was associated with a decrease in spontaneous viral clearance. Importantly, this effect seemed to be due to an interaction of CCR5-Δ32 with the recently described IL28B rs12979860 polymorphism, which has also been shown to influence spontaneous recovery from HCV infection. As IL28B polymorphisms are currently the strongest host genetic markers to predict treatment-induced clearance of HCV infection, we designed this study to examine for a possible interaction between the CCR5-Δ32 mutation and the two most predictive IL28B polymorphisms, rs8099917 and rs12979860, with respect to treatment-induced clearance of HCV infection.
Results

When compared to healthy controls, patients with chronic HCV infection had a significantly increased frequency of CCR5-Δ32 homozygosity (Table 1; p=0.039). The association was due to the European cohort, with an increase in CCR5-Δ32 homozygous subjects (2.9% vs 0.4%; p=0.0014) and a decrease in heterozygous subjects (11.3% vs 18.0%; p=0.0020). Further, the enrichment of CCR5-Δ32 homozygous European Caucasian subjects with CHC was quadruple that of what would have been expected based on the Hardy Weinberg equilibrium (p < 0.0001).

For treatment response to standard dual therapy (IFN-α/RBV), there was no significant difference between sustained virological responders (SVR) and non-SVR patients (NSVR) with respect to the frequency of CCR5-Δ32 genotype distribution or allele frequency (Table 2 and Figure 1).

In order to examine if the combination of the CCR5-Δ32 deletion with the IL28B SNPs, rs12979860 and rs8099917, improves prediction of treatment-induced HCV clearance, we used logistic regression modelling as previously described \textsuperscript{14, 21}. We found no evidence for any two-way interactions between either of the two IL28B SNPs individually or in combination with CCR5-Δ32 for NSVR, even though both the IL28B SNPs individually were significantly associated with treatment response (Table 3).
Discussion

The aim of this study was to establish whether there is an interaction between IL28B polymorphisms and CCR5-Δ32 with respect to predicting treatment-induced clearance of chronic HCV infection. Our results clearly show that A) CCR5-Δ32 does not directly influence treatment response of HCV genotype 1 to treatment with IFN-α and RBV and B) there is no interaction between IL28B genotypes and CCR5-Δ32 in this context. This is in line with previous, substantially smaller studies that have not shown a direct effect of CCR5-Δ32 on treatment response to IFN/RBV treatment \(^{21-27}\). As we have previously shown in a small cohort of patients, the outcome of IFN monotherapy, but not dual therapy, may be affected by CCR5-Δ32 \(^{21}\). The lack of a CCR5-Δ32 association in that and the current cohort may be due to a RBV effect. The impact of IL28B might not be masked by RBV, suggesting a much stronger role for variants of this gene in treatment outcome. Of note, age, viral load and gender were significantly different between responders and non-responders (data not shown; \(P<0.05\)). These clinical parameters are well known to be different between responders and non-responders \(^{28, 29}\).

It is well known that a major problem with clinical studies involving CCR5-Δ32 is that patient source does influence the CCR5-Δ32 frequency: CCR5-Δ32 has been mainly described in Caucasian populations and is subject to a strong Northern to Southern European decline in frequency \(^{30}\). Co-exposure to HIV, for which CCR5-Δ32 homozygosity confers a significant degree of resistance, may introduce another possible selection bias as HIV is transmitted via similar routes to HCV. Interestingly enough, CCR5-Δ32 was more common in the chronic HCV patients in Europe, similar to our published data \(^{12}\). Significant, but opposite, associations of CCR5-Δ32 with spontaneous recovery were identified in two cohorts, one East German the other Irish, both from single source outbreaks and with
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dependently and socioeconomically analogous backgrounds. This suggests that a weak (compared to IL28B) effect of CCR5-Δ32 on spontaneous recovery may be observed in genetically well defined contexts. More importantly, irrespective of these biases, the SVR and NSVR patients were subject to similar pre-selection criteria for therapy and despite this, CCR5-Δ32 did not impact on treatment-induced HCV clearance in our large multicenter cohort, nor was there a significant interaction between IL28B and CCR5-Δ32.

CCR5-Δ32 presents an attractive target for study in terms of HCV pathogenesis: HCV infection induces IFN-α and one of the interferon induced genes is CCR5. CCR5 is expressed on Th1 cells and facilitates the migration of T cells primed by antigenic molecules. It has been observed previously that in HCV patients, lymphocytes infiltrating the liver show an increased expression of CCR5. The presence of the CCR5-Δ32 deletion might result in a reduced expression of CCR5 in these patients, impairing their activation and migration to the infected liver. These mechanisms may be the reason why CCR5-Δ32 deletion may be involved in spontaneous clearance and possibly in response to IFN monotherapy. RBV, however, has been postulated to exploit a novel innate mechanism to potentiate the antiviral effects of IFN-α which could potentially also involve a bias favouring the Th1 immune response. In our multicenter cohort, the combination of RBV with IFN-α clearly overrode any potential effect of the CCR5-Δ32 deletion, whereas IL28B remained a strong predictive marker as previously reported.

In summary, CCR5-Δ32 did not influence treatment response to treatment with IFN-α/RBV and there was no significant interaction with IL28B genotypes. This suggests that CCR5-Δ32 is not relevant for treatment-induced clearance of HCV and that therefore CCR5 expression is likely not essential in this process. New powerful direct acting antivirals for difficult to treat HCV genotype 1 infection are currently being introduced and will likely reduce the impact of
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host genetics on treatment response \(^3^5\). In view of CCR5 inhibitors currently available for HIV treatment, this is an important observation, as our data suggest that neither genetic nor drug-induced impairment of CCR5 signalling is likely to impair the efficacy of anti-HCV therapy in patients with HIV/HCV co-infection. Indeed, a recent study in HIV/HCV co-infected patients reported no significant changes in viral titres or LFTs during a short term course of a CCR5 inhibitor \(^3^6\).
MATERIALS AND METHODS

SUBJECTS

Ethical approval was obtained from the Human Research Ethics Committees of Sydney West Area Health Service and the University of Sydney. All other sites had ethical approval from their respective ethics committees. Written informed consent was obtained from all participants. Characteristics of the study cohorts have been described elsewhere. Briefly, all treated patients were Caucasian and infected with genotype 1, the majority of these patients received pegylated interferon and ribavirin and had virological response determined 6 months after completion of therapy. The diagnosis of chronic hepatitis C was based on appropriate serology and presence of HCV RNA. Patients received therapy for 48 weeks except if there was less than a 2 log drop in HCV RNA after 12 weeks therapy. Patients were excluded if they were co-infected with HIV or HBV. The control data was obtained from previous studies done on healthy individuals from the same geographical region as the patients in this study.

GENOTYPING

The CCR5 Δ32 deletion was genotyped using high resolution melt, as described previously. IL28B data presented in this study have been previously published.

STATISTICAL ANALYSIS

The $\chi^2$ test or Fisher’s test where appropriate were used to examine differences in genotype and allele frequencies between SVR versus Non-SVR (NSVR), and chronic hepatitis C (i.e. NSVR plus SVR) versus healthy controls. P values less than 0.05 (two-sided) were regarded as significant. The relationships between CCR5-Δ32 and IL28B SNPs, rs8099917 and
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rs12979860, were investigated using logistic regression. Significance of all models was assessed by likelihood ratio tests. Analysis was carried out in R (v2.12).
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Conflict of interest: The authors declare that they have no competing financial interests in relation to this work.
References


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Figure 1.
Response in different CCR5-Δ32 genotypes (WT/WT = homozygous wildtype, Δ32/WT = CCR5-Δ32 heterozygous, Δ32/Δ32 = CCR5-Δ32 homozygous, all Δ32+ = all patients carrying one or two CCR5-Δ32 alleles.

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All patients</th>
<th>Australian patients</th>
<th>European patients</th>
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<tr>
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<td>Healthy Controls (n=836)</td>
<td>Chronic HCV (n=758)</td>
<td>Healthy Controls (n=168)</td>
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<tr>
<td>WT/WT</td>
<td>682 (81.6)</td>
<td>635 (83.8)</td>
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<td>Δ32/WT</td>
<td>150 (17.9)</td>
<td>108 (14.2)</td>
<td>30 (18.0)</td>
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<tr>
<td>Δ32/Δ32</td>
<td>4 (0.5)</td>
<td>15 (2.0)</td>
<td>1 (1.0)</td>
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</table>

Analysis: *WT = wildtype, numbers in brackets referes to %

<table>
<thead>
<tr>
<th>Allele</th>
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<th>European patients</th>
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<tr>
<td>WT</td>
<td>1514 (90.6)</td>
<td>1378 (90.9)</td>
<td>304 (90.5)</td>
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<tr>
<td>Δ32</td>
<td>158 (9.4)</td>
<td>138 (9.1)</td>
<td>32 (10.7)</td>
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* WT = wildtype, numbers in brackets referes to %
## Table 2

<table>
<thead>
<tr>
<th>CCR5 genotype</th>
<th>SVR (n=341)</th>
<th>NSVR (n=417)</th>
<th>Statistics</th>
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<tr>
<td>WT/WT</td>
<td>287 (84.2)</td>
<td>348 (83.5)</td>
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<tr>
<td>Δ32/WT</td>
<td>45 (13.2)</td>
<td>63 (15.1)</td>
<td>n.s.</td>
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<tr>
<td>Δ32/Δ32</td>
<td>9 (2.6)</td>
<td>6 (1.4)</td>
<td>n.s.</td>
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</table>

<table>
<thead>
<tr>
<th>CCR5 allele</th>
<th>SVR</th>
<th>NSVR</th>
<th>Statistics</th>
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<td>WT</td>
<td>619 (90.8)</td>
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<tr>
<td>Δ32</td>
<td>63 (9.2)</td>
<td>75 (9.0)</td>
<td>n.s.</td>
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</tbody>
</table>

* SVR = sustained virological responders, NSVR = non-SVR, WT = wildtype, numbers in brackets refer to %
CCR5-Δ32 and IL28B polymorphisms in treatment of HCV infection
<table>
<thead>
<tr>
<th>Value</th>
<th>European Patients (n=279)</th>
<th>Australian Patients (n=272)</th>
<th>Total Patients (n=551)</th>
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Note: The table represents the distribution of values for CCR5-Δ32 and IL28B polymorphisms in the treatment of HCV infection.
CCR5-Δ32 and IL28B polymorphisms in treatment of HCV infection